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Oral

Peptide and hyaluronic acid coated liposomes for targeting bacterial infections and sepsis.

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Abstract text
Introduction: Bacterial sepsis is a life-threatening condition that causes a global health burden associated with high mortality and morbidity. The problem has been exacerbated with the emergence of antibiotic resistance. Smart targeted biomimetic nanoparticles have emerged as a novel approach for enhancing the treatment of bacterial infections and sepsis. This study aimed to design vancomycin-loaded multi-functional liposomes.

Method: A novel (P3) peptide was designed using a data filtering technology (DFT) and synthesized, in silico study was performed to confirm binding between P3 and TLR4. HA-P3-Lipo was formulated using a thin film hydration technique. Characterization in terms of physical properties (size, polydispersity index (PDI), zeta potential), entrapment efficiency and drug release was done using Dynamic Light Scattering, RP-HPLC, and dialysis methods respectively. Biological stability and in vitro antibacterial activity were established via serum stability, hemolysis, MIC, antibiofilm and live/dead cells assay.

Results: In silico results confirmed strong binding between P3 and TLR4 with a binding energy of less than that of LPS. The size, PDI and zeta potential of HA-P3-Lipo were 121.9 ± 1.25 nm, 0.258 ± 0.02 and -6.69 ± 1.1 mV respectively, with 61.87± 2.1% encapsulation efficiency. In vitro drug release studies demonstrated sustained deliberation of VCM which was also reflected in the antibacterial activity. P3 ligand exposure was confirmed by degradation of the HA layer and charge switching from -25 to positive upon incubation with HAase. The antibacterial activity against methicillin-susceptible and resistant Staphylococcus aureus (MSSA and MRSA) revealed that HA-P3-Lipo had enhanced activity significantly, with a twofold reduction in the MIC. The antibiofilm study showed the superiority of HA-P3-Lipo with 39.6% Biofilm growth inhibition compared to 8.6% for VCM.

Conclusion and Significance: Multi-functional HA-P3-Lipo has shown significant potential to enhance the antibacterial activity of VCM and block the inflammatory cascade of sepsis which can improve therapeutic outcomes.

Analysis of biologics and nanoparticles in blood using asymmetrical flow field-flow fractionation (AF4) and selective detection

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Abstract text
The analysis and characterization of therapeutic proteins is crucial in order to ensure efficacy and patient safety. One aspect of these analyses is performed in the formulation to ensure, for instance, the oligomeric state and/or that larger aggregates are not present. An important question is, however, what happens to therapeutic proteins, after the they have been administrated (e.g. in the blood). In this presentation the fractionation of whole blood, plasma and serum is shown using asymmetric flow field-flow fractionation (AF4) with a minimum of sample pre-treatment. The analysis of therapeutic antibodies in blood plasma using AF4 with fluorescence, surface plasmon resonance (SPR) or mass spectrometry (MS) detection is demonstrated. In addition, analysis of nanoparticles and affibody-binding in plasma is shown. The results demonstrate the suitability and strength of AF4 for blood protein analysis and opens new important routes for the analysis and characterization of biologics and nanoparticles in blood and other biological fluids.

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AstraZeneca intro and glance into advanced formulations opportunities & challenges

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Abstract text

We will give a brief introduction to AstraZeneca’s areas of interest and footprint. Next, we will showcase some challenges and opportunities with new modalities, advanced formulations and drug delivery designed to provide patient centric products of the future supported by digital approaches.

Biologic drug delivery by inorganic fractal-like nanoagglomerates

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Abstract text
Introduction: Biologics (proteins, peptides, nucleotides) dominate the novel therapeutics market. A major obstacle in their employment, however, is their enzymatic degradation in vivo demanding high doses that result in side-effects. One way to avoid degradation of biologics is to encapsulate them in nanocarriers, such as lipid-based nanoparticles. An alternative approach is to use biocompatible inorganic nanoparticles, such as calcium phosphate (CaP). CaP nanoparticles have been explored as drug nanocarriers for biologics and as adjuvants in nanovaccines [1]. In this study, we aim to improve the drug loading capacities of biologics by utilizing inorganic CaP nanoaggregates made by flame aerosol technology, with fractal-like morphology and high specific surface area.

Methods: We use flame spray pyrolysis, a technique which allows tuning of NPs properties like composition, size, crystallinity [2]. These parameters are critical as they determine the mode of cellular uptake. We optimize the loading of CaP nanoaggreates with biologics [3], targeting two distinct applications: as antimicrobial peptide delivery carriers and as adjuvants for vaccination. The performance of the developed particles is benchmarked with the state-of-the-art lipid-based nanoparticles for biologic drug delivery.

Results: We have synthesized amorphous CaP NPs with varying silica content totune hydrodynamic size, biologic loading capacity, and cell cytotoxicity. We obtain a high specific surface area of greater 150 m^2/g for all these NPs. Upon loading ovalbumin, a model protein antigen, we achieved loading capacity values of up to 400 µg/mg NPs. None of these particles showed cytotoxicity on human lung epithelial cells. We further use ovalbumin loaded CaP NPs as a model system to optimize the antigen uptake and tune the inflammatory response by immune cells.

Conclusions/Impact: The flame-made CaP nanoaggrates developed on this study exhibit advantageous properties for their employment in biologics delivery with high loading values and minimal cytotoxicity.

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Biopharmaceutical approaches during long-acting injectable development

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Abstract text
During the past years, there has been an increased interest in Long-Acting Injectable (LAI) as a formulation platform, applied either for systemic or local administration, that may offer several benefits for patients. It may enable a steady release of drug over a period of weeks or months, a significant reduction of the frequency of administration may be possible, therefore reducing patient burden. Additional advantages can be the avoidance of first pass extraction, reduction of peak-to-trough ratios and the potential for higher bioavailability for compounds that do not readily absorb orally. During the past decade, a steady increase in marketed long-acting products was therefore recorded.

Despite being marketed for different therapeutic areas, their development is still complicated by various challenges related to product development, control over the in vivo release and tolerability and the design of long clinical trials. The aim of this presentation is to present an overview of the current status in biopharmaceutical profiling of LAIs from discovery to clinical development. The focus during discovery and preclinical development stages is to assess the potential suitability of new compounds for LAI development and the selection of formulation platforms. In addition, in vitro and in silico approaches to assess the impact of formulation parameters during clinical development will be presented. Ultimately, important gaps and challenges in the current approaches will be addressed as well. Future improvements in biopharmaceutical profiling could facilitate the overall drug development process of LAIs from discovery to clinical development stages: eg. by reducing animal studies, costs and overall timelines, guiding formulation design and understanding the mechanisms of underlying processes.

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Cationic Nanoparticles Modulate Electrostatic Interactions in Catabolic Cartilage

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Abstract text

Arthritic diseases are a leading cause of disability worldwide[1]. Current treatments result in unsatisfactory outcomes due to the lack of specificity, fast clearance, and inability to penetrate biological barriers. Advancements in nanotherapeutics have shown that electrostatic interactions and nano-sized particles are able to target and penetrate dense, negative tissues such as joint cartilage[2]. Nonetheless, clinical translation of functionalized drug carriers has been hampered in part due to a lack of understanding of the material's interaction with the pathological environment. It is important to consider the presence of aberrant proteolytic enzyme activity in the deteriorating tissue microenvironment during disease development. Utilizing an ex vivo enzymatic osteoarthritis (OA) model, our study investigates the interactions of polyamidoamine (PAMAM) and polyethyleneimine-coated (PEI) polylactic-co-glycolic acid (PLGA) NPs with cartilage extracellular matrix (ECM). ECM proteases including collagenase, hyaluronidase, and ADAMTS5 were used to mimic OA and reflect individual patient profiles. We demonstrate that the interactions between charged NPs and ECM biomolecules under catabolic conditions have implications for cartilage integrity, protein conformation, and immunomodulatory effects exerted by macrophages. Small, charged NPs can, via electrostatic interactions, stabilize GAG release and induce structural changes in enzymes such as ADAMTS5, as well as in enzymatic degradation products including aggrecan, and hyaluronic acid. Additionally, these interactions resulted in aggregate formations leading to differential expression of immunomodulatory signals. Studying NP-protein interactions in pathogenic conditions holds significant promise in driving the translation of NP applications to the clinic, ultimately revolutionizing personalized therapies, and overcoming the current barriers impeding the clinical translation of functionalized drug carriers.

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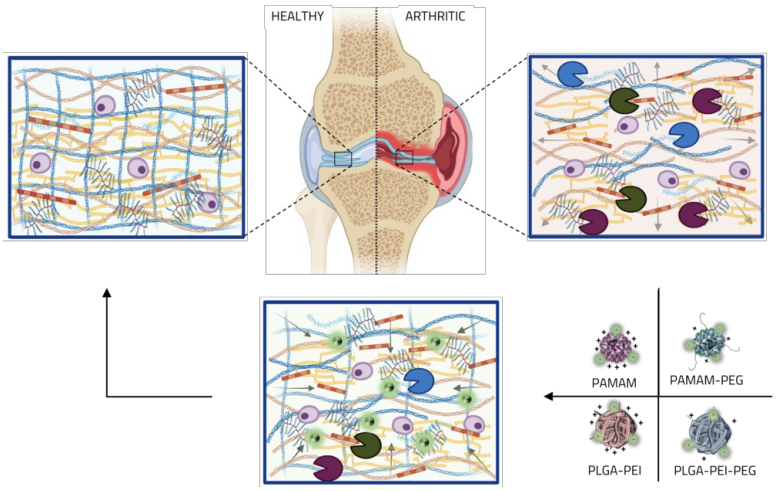

Development of a versatile temperature and humidity controlled sample environment for conducting SAXS measurements

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Abstract text

Protein-based drugs are among the complex and growing scientific topic in pharmaceutical technology. Proteins are extremely unstable to external changes, such as temperature and relative humidity. Dry powder formulations, such as lyophilized powder, offer a solution to maintain the stability of active ingredients in a drug product. However, proteins in dehydrated state not only lose their biochemical function but also undergo changes in dynamics (glass transition) and structure. Knowledge about protein structure at the molecular level in the solid-state and its transition upon rehydration is extremely important for improving formulation in the solid state form.

In collaboration with MAX IV we are developing a versatile, temperature and humidity controlled sample environment for conducting Small Angle X-ray Scattering (SAXS) studies on hydration of proteins, probiotics, amphiphiles and other soft matter systems. Using this sample environment, it would be possible to gain a deeper insight into the role played by the two most common factors that have profound influence on the stability of the native state of any protein of interest, namely, hydration and temperature. In addition, it will allow us to gain a better understanding of the influence of humidity on other biomimetic models for skin and lungs. The sample environment will be tested and commissioned at the CoSAXS beamline at MAXIV Synchrotron Facility.

Electroresponsive polymer brushes for non-invasive delivery of biologics

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Abstract text
Interfaces functionalized with polymers are known to provide excellent resistance towards biomolecular adsorption and for their ability to bind high amounts of biomolecules while preserving function and structure(1). However creation of a dynamic polymer interface that can switch reversibly between resisting and promoting binding has proven challenging. Most stimuli-responsive systems passively rely on a change of the surrounding solution to trigger a response. But the physiochemical environment in many biologic systems is static. Here we present the first interface that by electric signals can be actively switched between a high-capacity binding state (protein binding of <1 ug/cm2) to a completely non-fouling state (complete release)(2). The coating is re-usable meaning it is possible to load and release in multiple cycles. Release of bound biomolecules can be dosed by tuning the electric signal. Importantly, the electrode is fully operational when in direct contact with biological fluids and buffered environments. We anticipate that this technology could be applied for diagnostic purposes, capture and harvest, as well as controlled for on-demand in vivo delivery of biologics.

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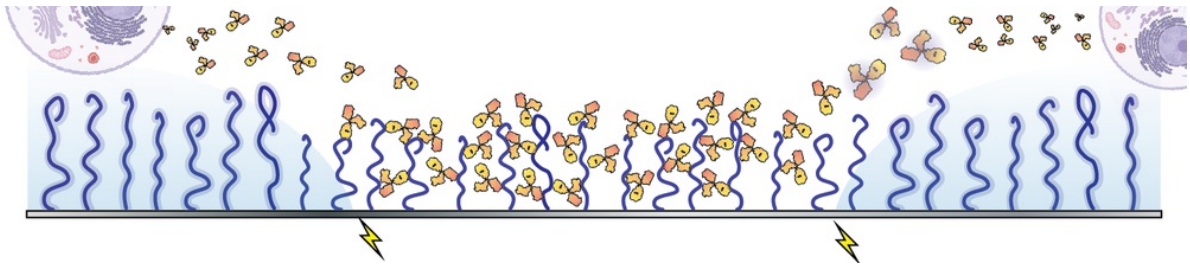

Evaluation of microfluidic in vitro method developed for behavior prediction of pharmaceuticals in subcutaneous tissue

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Abstract text

Subcutaneous administration is for many biopharmaceuticals the only viable route of administration. Still, we can today not fully predict the behavior and absorption profiles of subcutaneously injected pharmaceuticals with available *in vitro* methodology. We have previously developed a microfluidic-based *in vitro* method (MIS), to further help in these predictions by investigating interactions between drugs and polyelectrolytes of the subcutaneous tissue.[1]

Recently we used the method to study the interactions of seven pharmaceutical peptides with hyaluronic acid microgels. The results were quantified and compared with results from the SubCutaneous Injection Site Simulator “SCISSOR”, a commercially available method utilizing linear hyaluronic acid in solution to try to predict subcutaneous absorption profiles. The results from both methods were further compared with *in vivo* absorption, and bioavailability data from literature, aiming to establish *in vitro- in vivo* relationships.

The MIS and SCISSOR provided complementary information to better understand and predict transport and aggregation properties in a hyaluronic acid-rich environment and by extension certain behaviors *in vivo*. Quantitative relationships of transport rates through the microgel networks and SCISSOR matrix were seen. Further relationships between several parameters acquired from the MIS and time to peak plasma concentration *in vivo* were observed. Collected data from both the two *in vitro* methods were deemed suitable for inclusion into more comprehensive predictive *in silico* models.

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Formation of non-lamellar lipid nanoparticles and their use for biomolecular entrapment and delivery

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Abstract text

Non-lamellar lipid aqueous phases, such as reverse cubic or hexagonal phases, have increasingly been used to entrap biomolecules, both enzymes and other proteins. Both the curvature of the lipid aqueous interfaces in these phases, which determines the size of the aqueous cavities and hence the space given to the enzyme, as well as the interaction between the enzyme and the lipid layer are important factors that control the efficiency of the encapsulation. We have shown that mixtures of acylglycerides and acyldiglycerides can form highly swollen sponge phases (L_3) with aqueous pores up to 13 nm of diameter, which with the help of the dispersing agent polysorbate 80 (P80) form well defined nanoparticles in excess water [1]. These structures can be used to encapsulate a range of enzymes and other proteins such as Aspartic protease (34 KDa), Beta-galactosidase (460 KDa) and heme proteins for iron dietary supplements [2-4]. Size exclusion chromatography showed efficient encapsulation of both enzymes, yet they retained their enzymatic activity over months, surpassing the storage stability of pure enzymes in solution [2,3]. The reason for this can be understood in terms interaction of the enzymes into the lipid bilayer as shown by Raman spectroscopy [2] as well as by model studies using a lipid bilayer formed by spreading sponge phase particles on a supporting surface with QCM-D and neutron reflectometry [5]. The neutron reflectometry indeed shows that the enzymes penetrate the lipid bilayer [6]. This has been confirmed by neutron spin echo and molecular dynamics simulations [7].

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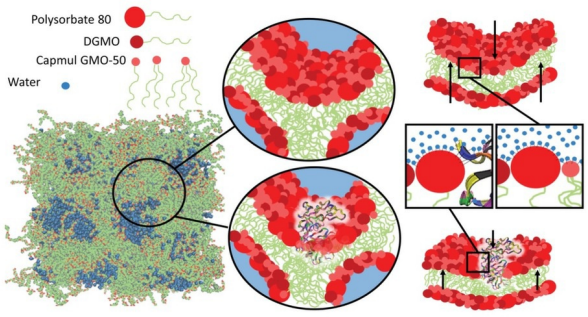

Figure: Location of Aspartic protease in a lipid sponge phase based on MD simulations. Adapted from [7].

Formulation and Characterization of Lipid Nanoparticles – Understanding Non-Viral Delivery

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Abstract text

During the COVID-19 pandemic the successful use of Lipid Nanoparticles (LNPs) for delivery of mRNA vaccines was highlighted. The same type of delivery system also has great potential for therapeutic gene editing. Gene therapies have traditionally relied on viral-based vectors for delivery, however such systems come with safety concerns related to their viral nature and can be challenging to produce in large quantities, resulting in high costs.[1] The benefit of non-viral systems, like LNPs, is that they can avoid many of the safety issues associated with viral vectors and decrease manufacturing costs.

In the Advanced Drug Delivery group at AstraZeneca we work on developing and understanding LNPs for a wide range of therapeutic indications. As LNPs are complex, they require in-depth knowledge of formulation, chemical stability, physicochemical and biophysical characterization, and how this relates to *in vitro* and *in vivo* results. Additionally, the scale-up of LNPs usually involves the change of formulations methods which adds another layer of complexity.

Herein we present some of the work that is currently being pursued in understanding and developing LNPs. This includes the use of Design of Experiments (DoE) to investigate how LNP formulation parameters relate to efficacy and stability, as well as how DoE can be leveraged when developing a scale-up process for LNPs. Additionally, we will present work on the physicochemical and biophysical characterization of LNPs, including the use Surface Plasmon Resonance, Small Angle X-ray Scattering, Cryo-TEM, and fluorescent probes which can be used to investigate how the formulation and composition of LNPs relates to structure and LNP-protein interactions.

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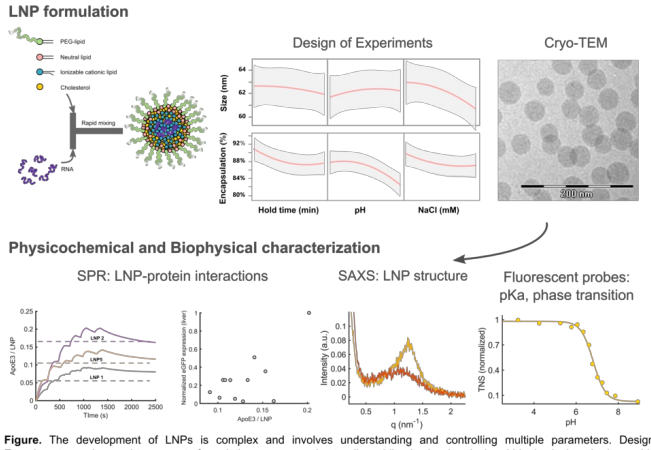

Figure. The development of LNPs is complex and involves understanding and controlling multiple parameters. Design of Experiments can be used to generate formulation process understanding while physicochemical and biophysical methods provides a more in-depth understanding of LNP properties.

Impact of non-ionic and anionic surfactant interaction on protein conformation

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Abstract text

Interactions between proteins and surfactants are important in a wide range of applications, including formulation of biologics (1). Theseinteractions are dynamic and reversible (2, 3), and addition of non-ionic surfactants has been shown to reverse SDS-induced unfolding (4-7). Nonetheless further understanding of the interaction landscape between protein and surfactant pairs is essential to enhance applicabilityof these systems. In this study, a model system consisting of human growth hormone (hGH), the non-ionic sugar surfactant dodecyl maltoside (DDM), and sodium dodecyl sulphate (SDS) is investigated with the intention to bring deeper insight to how surfactant pairs interact with proteins.

The primary method applied is titration fluorescence spectroscopy, with three different experimental designs; (i) exposing hGH to the surfactants individually, (ii) adding DDM to an SDS-hGH system, (iii) exposing hGH to mixed micelles. A deeper structural understanding is achieved through contrast variation SANS and ^{1}H NMR on samples at selected surfactant ratios.

One of the key results is the observed ability of DDM to interact with the SDS-unfolded protein. The current paradigm dictates that SDS interactions lead to protein unfolding upon complexation, while DDM hardly affects protein conformation of compactly folded proteins (3). However, our results clearly demonstrate that the non-ionic surfactant can interact with unfolded hGH as mediated by the presence of SDS.At the protein concentration used in this investigation, the co-adsorption of both surfactants onto the protein only happens in a narrow and well-defined window of DDM/SDS ratio. Further, when the DDM/SDS ratio is sufficiently increased, a gradual recovery of the conformational properties of the native state is attained. We have also been able to demonstrate that cooperative association occurs regardless of mixing order, which shows that the complexes formed are equilibrium structures.

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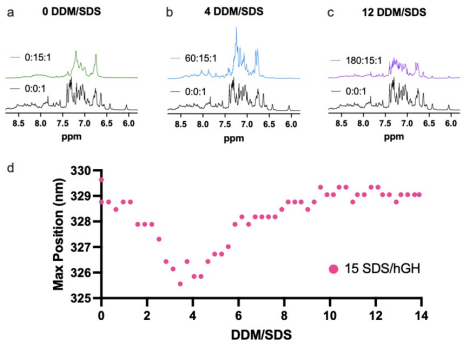

Figure. (a-c) partial ^{1}H NMR spectra of protein backbone region at different combinations of DDM and SDS, given in legend as DDM:SDS:hGH. All partial spectra include the native spectra. (d) evolution of maximum peak position (nm) of intrinsic protein fluorescence during titration of DDM to an hGH-SDS solution (15 SDS/hGH), as a function of DDM/SDS.

Inhalable porous particles as dual micro-nano carriers demonstrating efficient lung drug delivery for antibiotic and protein drugs

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Abstract text

Inhalation therapy treating severe infections is among the more complex and emerging topics in drug delivery and formulation. Micron-sized carriers are needed to deposit drugs into the lower airways, while nano-sized carriers are of preference for cell targeting. Here we present a versatile technology using spherical microparticles with excellent aerodynamic profile that dissolve in the lung fluid to ultimately generate nanoparticles enabling to enhance both extra- and intra-cellular bacteria (i.e., dual micro-nano inhalation strategy). The particles are composed by the condensation of nano-size amorphous silicon dioxide resulting in a disordered mesoporous silica particles (MSPs) with the optimal size for lung deposition (about 2.4 µm of diameter and narrow size span < 1). Clofazimine (CLZ), a potent antibiotic against multidrug-resistant tuberculosis, and lysozyme (LYS), the most abundant antimicrobial proteins in the airways, were used as study cases for small and biologics modalities. We developed a dry powder formulation using MSPs as sole excipient resulting in excellent aerodynamic performance (fine particle fraction <5 µm between 50-70 %). DSC, XRPD, SAXS and N_2 adsorption/desorption indicate that the molecules are confined in the nano-sized pores (9 – 10 nm) of the MSPs (shelf-life of 20 months at 4 ºC). Dissolution studies using simulated lung fluid showed *i*) a drastic dissolution enhancement of the poor-soluble CLZ (i.e., 16-fold higher than the free drug) reaching concentrations above the MBC against biofilms of M. tuberculosis, and *ii*) a sustained release of LYS maintaining high enzymatic activity (71-91%). The MSP carriers spontaneously degrade in simulated lung fluid into nano-sized drug carriers delivering high CLZ cargo inside macrophages (i.e., intracellular targeting). No measurable toxicity or impaired epithelial integrity was observed on macrophages and lung cells. This study presents a low-cost, stable dry powder formulation to efficiently delivers drug to the lungs overcoming technological and practical challenges for global healthcare.

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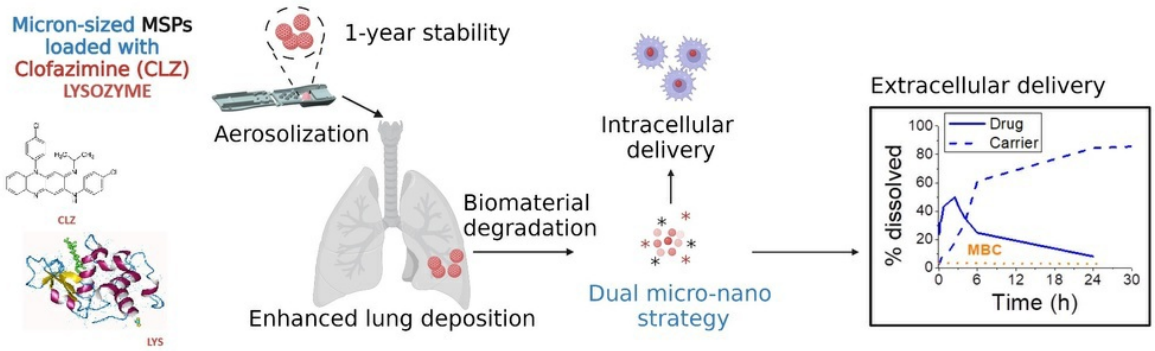
Micron-sized MSPs
loaded with
Clofazimine (CLZ)
LYSOZYME
CLZ
LYS
1-year stability
Aerosolization
Enhanced lung deposition
Biomaterial
degradation
Intracellular
delivery
Dual micro-nano
strategy
Extracellular delivery
% dissolved
Drug
Carrier
MBC
Time (h)

Molecular and Statistical Modeling of Polymeric Micelles

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Abstract text

Poor solubility of novel drug candidates represents a major obstacle during formulation development [1]. However, selecting the most suitable nanocarrier technology for a specific compound of interest is still largely limited to time- and resource-intensive experimental screenings. Gaining mechanistic insight through computational methods can help rationalize these approaches [2]. For the case of amphiphilic micelles based on poly(2-oxazolines) (pOx) and poly(2-oxazines) (pOzi), representing established drug carrier systems for anticancer agents [3], we exemplarily demonstrate the potential of molecular dynamics simulations and machine learning methods to complement experimental work and suggest novel formulations with optimized loading properties. All-atom simulations were performed on model compounds and different polymeric architectures in order to investigate the underlying drug-polymer interactions that enable ultrahigh drug loading (up to ~ 50 wt%) [4]. In addition, in-house data and available loading properties from multiple previous studies [5-7] were combined into an extended formulation database and subsequently used for statistical modeling of important solubilization metrics: loading capacity (LC) and loading efficiency (LE). Supervised machine learning methods based on various mixture-specific molecular descriptors were applied to develop a prediction tool that can be used by other researchers in order to evaluate the loading properties of these micellar structures for novel compounds.

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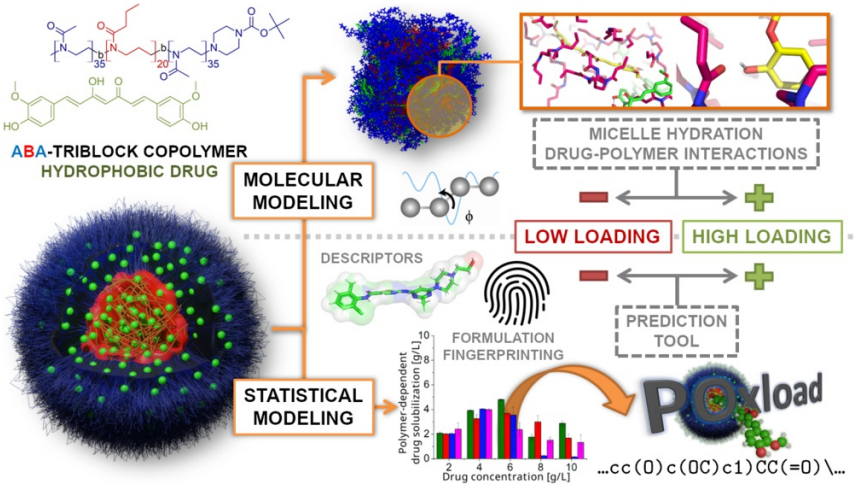

Mucosomes: innovative glycosylated mucin based nanoparticles as multi drug delivery platform

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Abstract text

Mucus is a complex barrier for pharmacological treatments and overcoming it is one of the major challenges faced during transmucosal drug delivery [1,2]. To tackle this issue, we introduce a novel class of glycosylated nanoparticles, named “mucosomes”, which are based on the most important protein constituting mucus, the mucins [3] Mucins are long polymeric glycosylated proteins composing the dense glycocalyx of mucosal epithelial cells or mucus layers covering the wet epithelia. In addition to protecting against shear stress and dehydration, mucins are also bioactive molecules towards microbes and mammalian cells [4]. Mucosomes were designed to improve drug absorption and residence time on the mucosal tissues. We have been able to synthesize mucosomes nanoparticles that are functionalized with glycans, and loaded with the desired drug in a single one-pot synthetic process. Using this method, we have been able to load a wide range of small, and macro, molecules with different physicochemical properties. Various *in vitro* models were used to test the mucoadhesive properties of mucosomes. *In vitro* and *in vivo* tests indicated that mucosomes did not induced adverse effects under the investigated conditions. We propose mucosomes as a ground-breaking nanosystem suitable for drug delivery. In particular, the ever-growing emergence of antimicrobial- resistant pathogens, demands innovative and transversal solutions. That is why mucosomes could be applied in several pathological contexts such as bacterial or fungal infections in mucus-related disorders.

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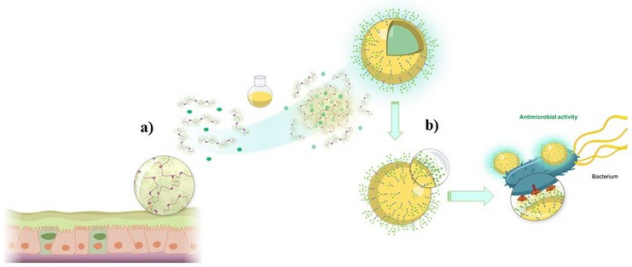

Figure 1

Stability of a IgG1 monoclonal antibody during deposition of controlled release coating using atomic layer deposition

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Abstract text

Introduction: There is a need within the biopharmaceutical market to administer highly concentrated monoclonal antibody (mAbs) solutions, to minimize the number of injections needed during treatment. However, these highly concentrated solutions tend to be very viscous and gel-like, rendering them impossible to inject [1]. Nanexa's PharmaShell® technology for long acting injectables is a promising solution to this issue, as it allows for highly concentrated suspensions as well as possible storage stability benefits [2]. The aim of this study was to verify the feasibility of utilizing the PharmaShell® technology for coating monoclonal antibodies.

Methods: Buffer replacement was performed for a model IgG1 mAb ATH3G10 before particles were spray-dried with trehalose and histidine as stabilizing excipients. Thereafter, powder was coated with an inorganic controlled release coating in a patented deposition process performed using the ALD process to apply a nanometre-thin coat of aluminium oxide on each particle. Affinity of the mAb was analysed with ELISA before and after coating and aggregation propensity was analysed using UV-absorbance spectroscopy. Morphology was analysed using scanning electron microscopy. Injectability using a texture analyzer was studied after suspending particles in aqueous diluent.

Results: ALD coating of the model mAb does not affect morphology, affinity and aggregation propensity significantly. Coated material could be suspended up to 250 mg mAb/mL with an injection force < 15 N.

Conclusion: This study demonstrated unchanged affinity and aggregation propensity when applying a controlled release coating onto a spray-dried monoclonal antibody. Thus, it could be verified that it is feasible to utilize the PharmaShell® technology for monoclonal antibodies and that highly concentrated suspensions can be prepared with an acceptable injection force. Next step of this project is to study the pharmacokinetic properties prolonged release coated ATH3G10 after subcutaneous injection in a preclinical model.

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Structure and properties of proteins in solid-state formulations and the effects of carbohydrate excipients

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Abstract text

The stability of biologically derived pharmaceuticals constrains their applications, and solid-state formulations, often obtained through lyophilization, offer a solution to this problem. However, our understanding of protein structures in the amorphous solid state and their behavior during rehydration is limited.

During the past few years in NextBioForm consortium we performed several synchrotron small and wide angle X-ray scattering (SAXS/WAXS) studies to characterize the structure of a model protein, lysozyme, in the solid state and its structural transition upon rehydration to the liquid state. Moreover, the effects of temperature and addition of carbohydrate excipients was studied using SWAXS, DSC and other techniques.

The results show that the lysozyme undergoes distortion upon drying to adopt structures that can continuously fill the space to remove the protein-air interface that may be formed upon dehydration[1]. Above a hydration threshold of 35 wt%, the native structure of the protein is recovered. The evolution of SAXS/WAXS peaks upon change of water content in a broad range of concentrations provides further insights into the structural changes in the protein.

Analysis of thermal behavior of lysozyme using several experimental methods showed three distinct unfolding behaviors at different water contents[2]. In the strongly dehydrated state the unfolding is an irreversible process and can be described by a kinetic approach; at high water contents the process is reversible, and the thermodynamic equilibrium approach can be used. In the intermediate range of water contents the system is phase separated and the thermal denaturation involves two processes: melting of protein crystals and unfolding of protein molecules. Addition of carbohydrate excipients such as sucrose, changes the behavior describe above: according to SAXS data, the protein molecules in the solid state recover their native structure observed in liquid[3].

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Wildlife - a new horizon for therapeutics and drug delivery

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Abstract text
Wildlife comprises a diverse group of animals and delivery of drugs to these populations is challenging. There is a growing need for more research into therapeutics for wildlife species and the COVID-19 pandemic has highlighted zoonotic disease and made us acutely aware of the important link between human and wildlife health. There are considerable challenges to be overcome for the successful treatment of wildlife including the need for species-specific pharmacokinetic studies and tailored drug delivery for these specialist animal populations[1]. The objective is to highlight the area of wildlife therapeutics as an emerging field by presenting two research projects from Aotearoa New Zealand.

The yellow-eyed penguin (hoiho, *Megadyptes antipodes*) is one of the most endangered penguin species in the world. Avian malaria is a significant threat to the survival of hoiho. The antimalarial drug malarone is administered to yellow-eyed penguins, however there have been no pharmacokinetic studies of malarone in the yellow-eyed penguin and the dose given is based on the human dose. We have found that penguin pharmacokinetics is distinct from even other avian species and so there is a critical need for species-specific pharmacokinetic data to guide effective dosing and improve wildlife health and conservation.

The second study is designing delivery systems for the brushtail possum, the most significant vertebrate pest in New Zealand. Disrupting fertility is the most humane method of control. D-Lys^6-GnRH is a water-soluble analogue of gonadotrophin-releasing hormone (GnRH), a peptide essential to reproductive function. We formulated D-Lys^6-GnRH into polymeric nanoparticles with high encapsulation efficiency (95 ± 4.1%) and following i.v. administration, serum levels of luteinizing hormone increased within 15 min and was dose dependent. Importantly, D-Lys^6-GnRH-loaded nanoparticlesresulted in a significant biological response to reduce fertility *in vivo.* We have demonstrated that nanomedicines have application for the delivery of biocontrol agents to wildlife.

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Poster

1 - Medium-chain Fatty Acid Permeability in the Fasted State

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Abstract text

Medium-chain fatty acids (MCFAs) have gained significant attention due to their potential applications in various industries, including pharmaceuticals, food, and cosmetics. In this study, we carry out a series of computational experiments to understand the permeability and synergistic effects of a series of medium-chain fatty acids. We are employing molecular dynamics simulations to explore the interactions of MCFAs with lipid bilayers at the molecular level. We have in previous simulation studies (Holmboe et al., 2016) seen effects on the free energy of solvation for small molecular drugs from differences not only in the composition of the lipid bilayer but also when bile salt is present in the part of the simulation cell that represents the luminal part of the intestine. Here we hope to probe the significance of such effects on the series of MCFAs, paving the way for more knowledge-based design of pharmaceutical dosage forms that involve MCFAs for increasing permeability. Our results include *in silico* determinations of permeability rates for MCFAs, in isolation as well as at higher concentrations and finally taking into account the presence of the bile salts as potential permeability modulators.

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2 - Sucrose versus Trehalose: A Broad Study Using Molecular Dynamics Simulations

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Abstract text
Sucrose and trehalose are widely used preservatives for various applications in pharmaceutical, food
and cosmetic industries[1]. In this work we have performed comparative studies for the broad range of
concentrations of both sugars in water, using models published in the paper by K. Ahlgren et al.[2]
Simulated systems were of two kinds: the ones which were simulated only at the 298 K and the ones
which underwent pre-heating (annealing)[3] at 450 K and then cooling to 298K before production runs
under 298 K. We discovered significant effects of both hydration and pre-heating on behaviors of both
sugars. The obtained results can help in selecting either sucrose or trehalose for a particular application.
Such selection shall be based on the knowledge of their hydration and glass transition behaviors at
particular concentrations.

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3 - Machine Learning Assisted Optimization of Magnetic Hyperthermia

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Abstract text
Background: Superparamagnetic iron oxide nanoparticles (SPIONs) have shown promising clinical applications due to their capacity to release heat in an alternating magnetic field and increase tumor cell sensitivity to chemotherapy. Despite advancements in the field, the relationship between SPION properties and hyperthermia efficacy remains complex and not fully understood.

Aim: To enhance comprehension of the relationship between the physicochemical and magnetic properties of SPIONs and their hyperthermia efficacy using a machine learning (ML) approach.

Method: Starting with 19,378 articles, a procedure that combined natural language processing with manual curation was applied to produce a dataset containing 399 data points across 15 physicochemical and magnetic properties. To promote normal distribution, five data transformations were explored. Subsequently, the performance of 10 ML models was assessed, including Support Vector Regressor (SVR), Random Forests, and Neural Networks. Ten-fold cross-validation was utilized to assess the model's performance and selection. The significance of individual features in the top-performing model was measured using Permutation Importance.

Results and Discussion: The SVR model displayed the most promising results with an R-squared of 0.98 and a Mean Absolute Error of 25.7 when predicting SPION's Specific Absorption Rate (SAR). The Box-Cox transformation was the most effective strategy for enhancing the SVR model performance. The external magnetic field applied was the most significant predictor feature, with a permutation importance value of 2.12, followed by saturation magnetization and core diameter.

Conclusions: The ML approach, especially the SVR model, successfully enhanced our understanding of the relationship between SPION properties and hyperthermia performance. Moreover, the relationships between the Specific Absorption Rate value and the predictor features were identified as predominantly non-linear. This insight underscores the importance of ML models to capture these complex relationships accurately. Future research should prioritize expanding the dataset size and focusing on these pivotal features for more robust and comprehensive insights.

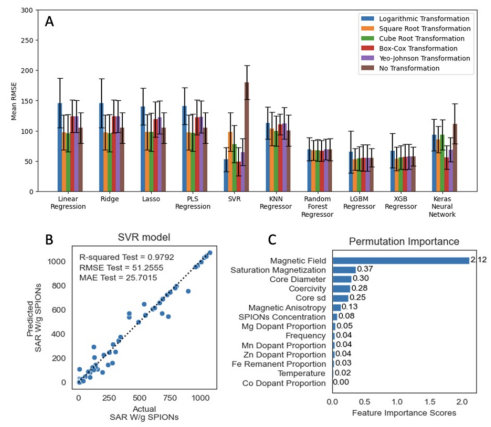

Figure 1. A: Ten-fold cross-validation model performance after hyperparameter tuning. B: Support Vector Regressor model performance. C: Feature Importance of magnetic and physicochemical SPION's features.

4 - Simulations Unveil Fate of Biologics and Enhancers

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Abstract text

The utilization of permeation enhancers (PEs) as excipients has emerged as a promising strategy for facilitating the oral delivery of biologics [1]. Effective co-localization of high concentrations of macromolecules (MMs) and PEs at the epithelial surface is crucial to enhance the bioavailability of biologics. However, the distribution and arrival timing of these two groups of molecules in the small intestine are significantly influenced by intestinal motor activities.

In the gastrointestinal tract, two primary motor activities, peristalsis and segmentation, play a dominant role in intraluminal transport processes. Intestinal peristalsis occurs during various phases of the migrating motor complex, while segmentation predominantly takes place in the fed state. The distinctive characteristics of these motor activities introduce complexities in the context of oral delivery of MMs and PEs [2].

Variability in motility intensity, pocket size, contraction level, release location, and the composition of intestinal fluid significantly impact the intraluminal distribution and the time of arrival at the epithelial surface. To comprehensively describe these movements, mathematical modeling of these motor activities is conducted based on magnetic resonance imaging videos. Subsequently, computational fluid dynamics simulations are employed to model intestinal fluid flow in conjunction with drug transport driven by the movements of the gut wall. These simulations unveil the fate of drug molecules influenced by intestinal motility (see the figure).

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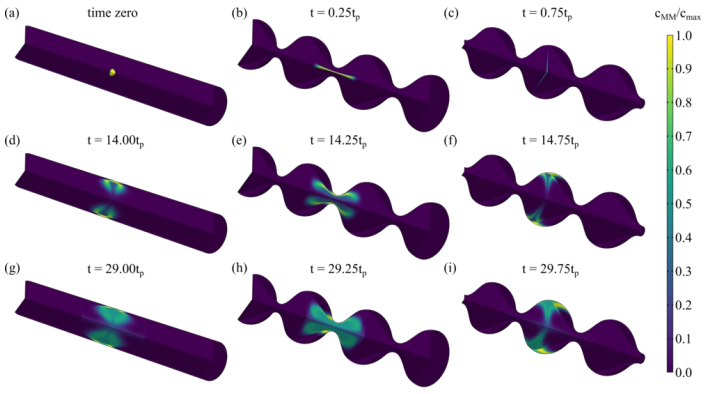

Three-Dimensional Representation of Normalized Insulin Concentration During Segmental Motility. Panel (a) presents a spherical concentration as the initial condition for a MM within a 2 mL pocket, with a period time of tp = 3s. Cases (b–i) exhibit MM distributions at various timepoints.

5 - Amorphous solid dispersions studied with molecular dynamics simulations

Xuezhi Zhuo[1] , *Vito Foderà[1], Per Larsson[2], Christel A.S. Bergström[2], Korbinian Löbmann[1, 3],* ***Aleksei Kabedev***[2]

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Abstract text

This research offers a comprehensive exploration of the stabilization of amorphous solid dispersions (ASDs) utilizing protein excipients and leverages molecular dynamics (MD) simulations to provide a unified understanding of this critical pharmaceutical domain.

Investigating the interplay between drug loading and stability in protein-stabilized ASDs, we utilized β-lactoglobulin as a model protein and various drug molecules, including indomethacin. Below a specific drug loading threshold (40-50%), single-phase ASDs exhibited a single glass transition temperature, while exceeding this threshold introduced a secondary transition temperature. MD simulations unraveled pivotal stabilization mechanisms, including reduced drug molecule mobility in the first drug shell and the formation of hydrogen-bond networks, predominantly involving glutamic and aspartic acids on the protein surface. These mechanisms aligned with experimental data, underlining their significance in stabilizing ASDs, particularly under dry storage conditions.

In another series of experiments five additional drug molecules, including those lacking hydrogen bond donors, were examined in protein-stabilized ASDs. Experimental techniques and simulations were combined to determine maximum stable drug loadings. The results demonstrated that certain drugs remain stable at high loadings, while others have defined limits. Stabilization mechanisms included steric confinement and hydrogen bonding at low loadings (≤40%), with inter-drug hydrogen bond networks, ionic interactions, and high glass transition temperatures becoming factors at higher loadings.

This work extends beyond stability analysis to explore the broader capabilities of MD simulations. It delves into controlled drug release from ASDs and the mechanisms influencing it. We investigate drug delivery to plasma membranes and the solubility of active pharmaceutical ingredients (APIs) carried by ASDs, all through the lens of MD simulations combined with experimental work.

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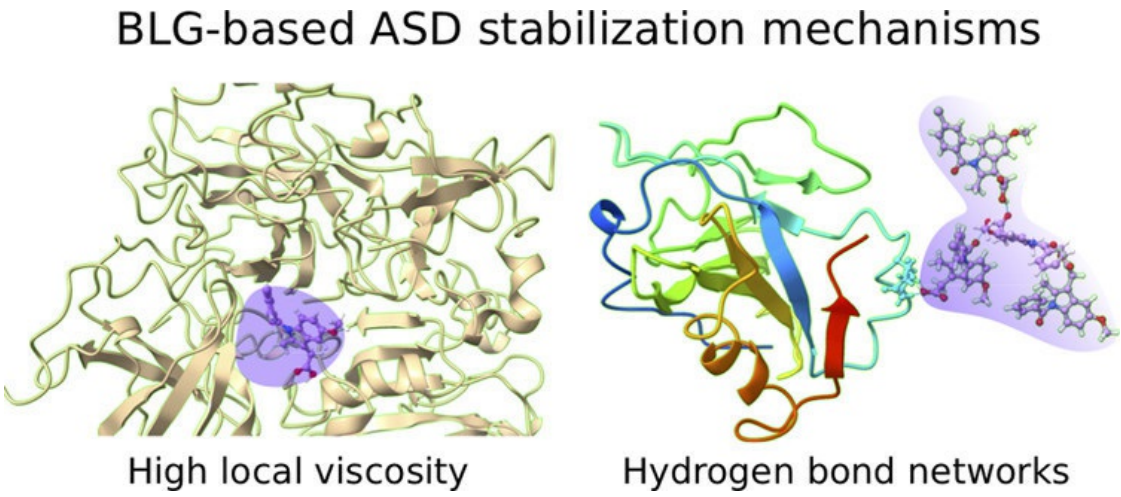

6 - How does data quality and data quantity impact prediction of solubility?

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Abstract text

Solubility is one of the most important physiochemical properties of drug molecules. However, the prediction of solubility remains a challenge. One of the main reasons is the lack of high-quality large data sets.[1] In this study, the impact of data quality and quantity on solubility prediction was investigated based on Janssen in-house solubility datasets.

The datasets were washed by making use of three different approaches: i) measured or experimental log D, ii) inclusion or exclusion of amorphous residue and iii) adopting or not undertaking a quality check. This resulted in the extraction of four datasets with different level of noise and size: dataset Clean (n=1658), dataset Noisy (n=5044), dataset Pred_logD (n=2598), and dataset Amorphous (n=4007).

Two experiments are performed on the datasets. For the first experiment, random forest regressors using 18 RDkit descriptors as input features were trained and evaluated via nested cross-validation. Outer loop and inner loop scores show that the model trained with dataset ‘Clean’ gives the best performance. In the second experiment, 10 training and evaluating processes were performed on each of the four datasets. For each process, a clean intrinsic solubility test set was randomly split from dataset ‘Clean’ and the compounds in the test set were removed from the current studying dataset. A model was then trained on the remaining compounds and the performance was evaluated via the test set. Models trained with dataset ‘Clean’ gave the best performance but did not come out as significantly superior compared to the nosier datasets.

In conclusion, models trained with a small, clean, and diverse dataset were providing acceptable predictions. Model trained with noisy but much larger dataset were herein able to provide comparable prediction qualities. However, further investigation is required to increase the number of descriptors, as the descriptors used might not be enough to predict solubility.

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7 - Mechanistic modelling of subcutaneous administrations: comparison of approaches

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[2] Pharmetheus AB

Abstract text
The subcutaneous (SC) route has been used to administer drugs to patients for over 150 years. This route has been used for the delivery of a wide spectrum of therapeutic substances. In recent years, the route has become preferential for biologics that are usually administered intravenously [1]. Combining mechanistic models for SC administration with physiologically-based pharmacokinetic (PBPK) modelling allows for prediction of bioavailability, translation from preclinical to clinical, and extrapolation to special populations, supporting decision making and reducing costs and animal usage.

Here, the SubQ model was evaluated for the absorption of small molecules administered SC to different species. This was compared to a standard modelling approach in PK-Sim.

All activities were performed within MoBi and aligning to implementations in PK-Sim to allow for full integration in the Open Systems Pharmacology framework [1]. A previously described model for local distribution and absorption after SC administration was used as starting point [2]. The SubQ model included 99 layers of intracellular and extracellular compartments and distribution to blood and lymph vessels, describing the diffusion of the compound into tissue surrounding the injection site. Systemic concentrations after SC administration were simulated based on the administered dose, dose volume and compound characteristics.

Two approaches were compared: With standard modelling approach, the SC administration was added to the interstitial skin compartment in the PK-Sim model. With the SubQ approach, the SC administration occurred in the SubQ model, which drains from interstitial to the plasma and through lymph to the plasma of the conventional PBPK model. Both simulated plasma concentrations were compared to observed data from literature. Several compounds and species were simulated.

Standard modelling resulted in quick absorption to the plasma, whilst the SubQ simulations resulted in a slower absorption to the plasma. Slower absorption was in line with the observed data from literature.

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8 - Polymer Substitution of Porcine Artificial Colonic Mucus Optimization for Drug Diffusion Study

Marco Tjakra[1] , *Nopdanai Chakrapeesirisuk[1], Magdalena Jacobson[2], Jens Eriksson[3], Mikael Sellin[3], Alexandra Teleki[1], Christel Bergström[1]*

[1] The Swedish Drug Delivery Center, Department of Pharmacy, Uppsala University, BMC P.O. Box 580, SE-751 23, Uppsala, Sweden.

[2] Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, P.O. Box 7054, SE-750 07, Uppsala, Sweden.

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Abstract text

Aim:

To select appropriate polymer for porcine artificial colonic mucus (PACM) formulation which captures diffusion properties of native porcine colonic mucus.

Method:

Biosimilar PACM prototypes with focus on polymer selection were generated to mimic Porcine Native Colonic Mucus (PNCM) and to improve previous model of PACM with polyacrylic acid (PAA). PACM viscosity & storage modulus were measured with rheometer ARES-G2. Surface charge and zeta potential were determined using Dynamic Light Scattering (DLS). Binding coefficients of model compounds were calculated using Microscale Themophoresis (MST). Then diffusion coefficient were analyzed by using particle tracking resulting in Mean Squared Displacement (MSD) and using Fluorescence Recovery After Photobleaching (FRAP). Permeability studies of model drugs (Atenolol, Ibuprofen, and 4K neutral FITC-Dextran) were performed in a Transwell setup, applying artificial colonic mucus on top of the filter and running the experiment from the donor to the receiver chamber. Samples were withdrawn at predetermined time intervals and analyzed with UPLC-MS.

Results and Discussion:

PACM with PAA was used as the benchmark for developing prototypes of PACM by selecting different polymers backbone, namely hydroxyethylcellulose (HEC), hyaluronic acid (HA), sodium alginate (SA), and pectin (PEC). Among these formulations, gelling ability was visually observed and apparent viscosity was measured within the range of 10 to 1000 Pa.s and within 10^{-4} to 10^{-2} MPa for storage modulus[1]. From measurements of zeta potential, the PNCM is negatively charge with zeta potential value around -21.9 mV and the closest polymer formulation for this value is PACM based on HEC (-19.4 mV). Binding coefficient of FITC-Dextran 4K+ with PNCM shown to be closer to the PACM HEC compared to PACM PAA.

Final conclusion

PACM HEC is established as a substitute of PACM PAA with closer properties to PNCM. Evaluation of PACM HEC

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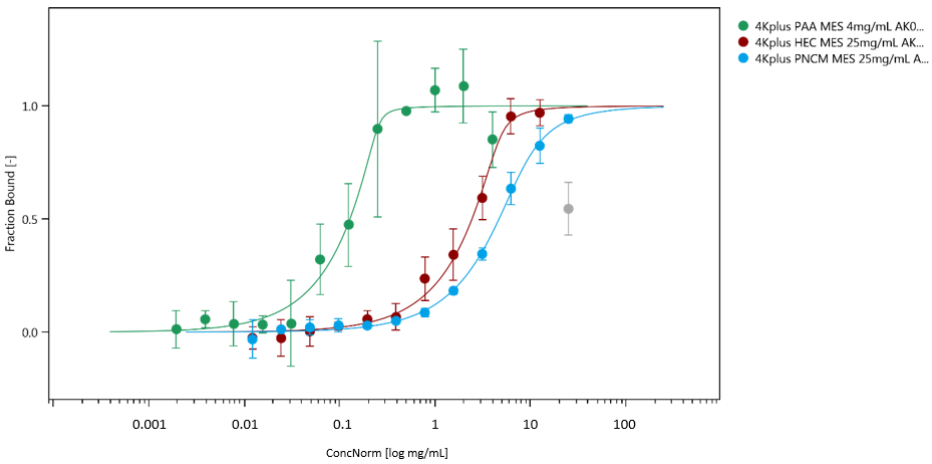
4Kplus PAA MES 4mg/mL AK0...
4Kplus HEC MES 25mg/mL AK...
4Kplus PNCM MES 25mg/mL A...
Fraction Bound [-]
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
ConcNorm [log mg/mL]

9 - Leveraging enzymes to overcome the gastrointestinal mucus barrier

Marilena Bohley[1] , *Angela Steinauer*[2], *Jean-Christophe Leroux*[1]

[1] Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

[2] Institute of Chemical Sciences and Engineering, Ecole polytechnique fédérale de Lausanne, Switzerland

Abstract text

Oral delivery of peptide-based drugs faces significant challenges due to limited absorption in the small intestine caused by gastrointestinal (GI) barriers comprising mucus, epithelial barriers, and digestive processes.[1] Despite numerous formulation efforts to enhance oral bioavailability, clinical success has been limited, with commercial peptide drugs achieving bioavailability rates of around 1%.[1,2] While many approaches focus on improving drug permeability through the intestinal epithelium, the mucus barrier has remained largely untapped. Mucus is a thick hydrogel composed of mucins (MUC), highly glycosylated proteins, primarily responsible for trapping and clearing pathogens while simultaneously impeding macromolecule permeability.[3,4] We hypothesized that targeted degradation of gel-forming mucins in the small intestine can reduce the barrier properties of mucus. Inspired by the mechanisms that allow bacterial pathogens to overcome the GI mucus barrier, we employed mucin-specific proteases (mucinases) to degrade GI mucus.[5] These were tested for their potential to reduce the viscosity of porcine small intestinal mucus. The best performing candidate, StcE[6], a bacterial protease from *Escherichia coli* was thoroughly investigated. Using a plate-and cone rheometer, we found that 6 µM StcE reduced both the viscous (G'') and elastic (G`) properties of mucus by more than 50%. Maximal effects were reached within 5 min after incubation. In a next set of experiments, fluorescence recovery after photobleaching (FRAP) was used to demonstrate that StcE can increase the permeability of Cy5-labelled poly(ethylene glycol)s (1 and 5 kDa) through mucus. StcE significantly increased the proportion of fully mobile molecules while simultaneously decreasing the proportion of the immobile fraction. Overall, we demonstrate that StcE can be leveraged to reduce barrier properties of GI mucus and enhance the permeability of macromolecules. StcE has the potential, especially in combination with other delivery strategies, to improve macromolecular drug absorption. This research was supported by the ETH Zurich Postdoctoral Fellowship program.

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10 - The impact of granule fragmentation on tableting performance of dry granulated particles

Maryam Tofiq[1] , *Göran Alderborn[1], Lucia Lazorova[1], Josefina Nordström[1], Ann-Sofie Persson[1]*

[1] Uppsala University, Department of Pharmaceutical Biosciences, SE

Abstract text

The degree of fragmentation and deformation of dry granulated particles have been reported to affect the compression behavior and thus, the final tablet tensile strength (σ_t) [1,2]. However, a thorough investigation on how the fragmentation behavior affects the tableting performance i.e. σ_t is missing. Therefore, in this study the granule fragmentation behavior is visually investigated and related to the σ_t. To accomplish this, the impact of granule variations such as shape and size was avoided by using isodiametric mini-tablets as surrogate granules. These consisted of microcrystalline cellulose (*MCC*), α-lactose monohydrate (*LAC*), and a systematically variated mixture of them at different proportions. The isodiametric mini-tablets were 3 mm. The granules were then compressed at different compression pressures to cover different strain points, which were selected to reflect the compression processes defined in our previous paper [3]. The compacted granules at respective strain points were ejected and gently crumbled into single granules. These were subsequently inspected for fragmentation and deformation behavior with light microscopy and dry powder laser diffraction. This was then linked to the σ_t attained at a tablet pressure of 300 MPa. The granules tended to fragment into three size ranges i.e. breakage size, splitting size, and disintegrating size (fine size). The granules that consisted only of *LAC* had the highest degree of fragmentation and thus, disintegrating sized particles. Their deformation consisted of a combination of chipping, breaking, disintegration, and finally plastic deformation. Further, their low intergranular bonding forces (due to the low degree of granule plastic deformation) gave tablets of low σ_t. The highest σ_t was obtained for the granules consisting of a combination of brittle and plastic material. Thus, a combination of particle fragmentation and plastic deformation is required to form tablets with a sufficient σ_t from dry granulated particles.

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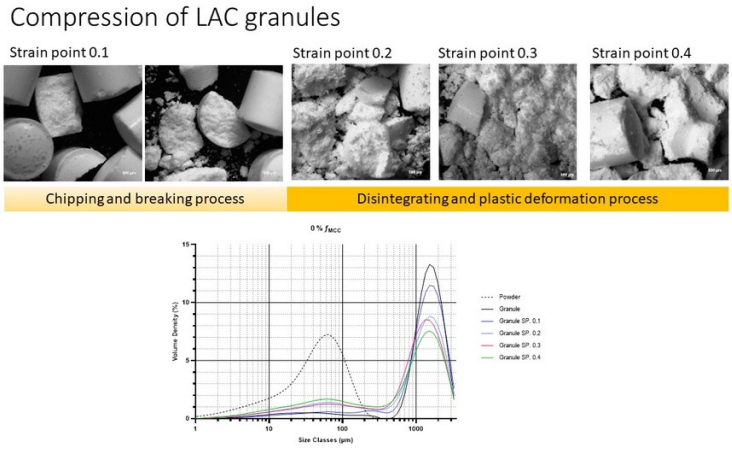

11 - Artificial vitreous in development of long-acting injectables for intravitreal administration

Linda Wirman[1] , *Tatu Assmuth*[1], *Ville Pollari*[1], *Mika Jokinen*[1]
[1] DelSiTech Ltd, Finland

Abstract text
Due to the increasing number of age-related retinal diseases intravitreal injections are becoming more common. Monthly or bimonthly injections are common to achieve optimal efficacy, and repeated injections may cause complications. Hence, there is a need for long-acting injectables to lengthen the treatment interval. DelSiTech has developed injectable depots for extended release of active pharmaceutical ingredients (API). The technology is based on amorphous silica (SiO_2). APIs are embedded in silica microparticles, which together with silica nanoparticles and water form an easily injectable hydrogel. The dissolution rate of silica and release rate of API can be adjusted from days to years.

Three artificial vitreous hydrogels were designed to evaluate the properties and behavior of the silica-based injectable depots in intravitreal injections. They mimic the rheological properties of the native vitreous hydrogels, and the components were mainly similar as those in the native vitreous, i.e., hyaluronic acid, glucose, water, typical salts of the vitreous, and agar as a substitute for type 2 collagen. The mixture was gelled inside a transparent plastic sphere simulating the eye. The rheological properties of the three different artificial vitreous hydrogels range from the typical values of rabbit eyes to pig and human eyes, which also include age-related changes in the rheological properties of the human eyes.

The three artificial vitreous hydrogels were used to visually study how the injectable silica-based depots with varying rheological properties behave during and after injections. Silica microparticle suspensions were used as controls to simulate the depots in a non-gelled structure. Clear differences were observed both in injections and in the behavior of the injectable depots in the different artificial vitreous hydrogels. The visual inspections in the artificial vitreous hydrogels were also compared to the fundus images taken from rabbits in an in vivo study conducted with the same injectable depots.

12 - 3D COLONOIDS AS COLONIC IN VITRO ADME MODELS REFLECTIVE OF NATIVE EPITHELIUM

Rebekkah Hammar[1] , *Patrik Lundquist*[1], *Daisy Hjelmqvist*[1], *Jens Eriksson*[2], *Maria Letizia di Martino*[2], *Ana Lopes*[2], *Wilhelm Graf*[3], *Mikael E. Sellin*[2], *Per Artursson*[1]

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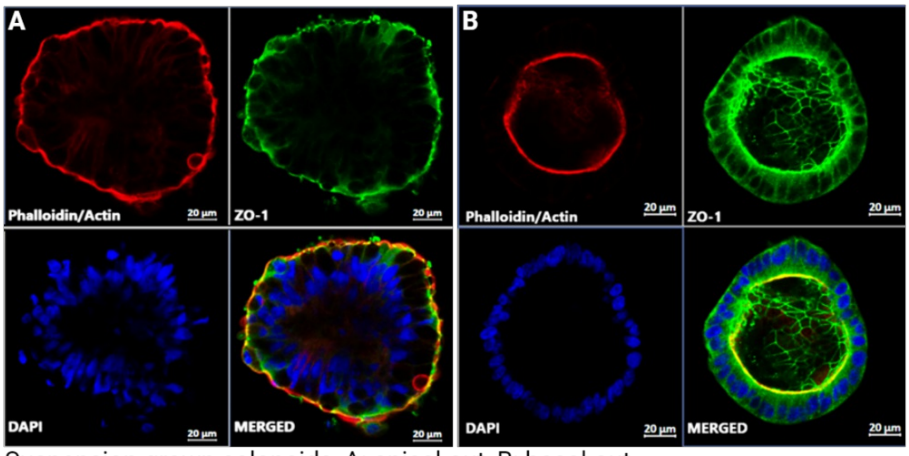
[2] Uppsala University, Department of Medical Biochemistry and Microbiology

[3] Uppsala University, Department of Surgical Sciences

Abstract text

With colonic inflammatory disorders on the rise globally, the need for *in vitro* assessment of human colonic drug absorption, distribution, metabolism, and excretion (ADME) is growing. Historically, immortalized *in vitro* cell models such as Caco-2 cells have been used. Such models inadequately reflect native ADME protein levels and the various colonic epithelial types, of which goblet cells play a key role. To address both of these issues, we generated apical- and basal-out suspension-grown 3D colonoids from healthy human donor colonic stem cells. Native-like ADME protein expression levels and the presence of goblet cells were confirmed using global and targeted proteomics, supported by immunohistochemistry staining. Colonoid barrier function were assessed by live confocal fluorescence microscopy and fluorimetry, confirming tightness and indicating high efflux activity. Phase I and II colonic metabolic activity was measured using probe substrates. Further functional characterization was performed by determining the intracellular bioavailability (F_{ic}) of a drug panel with known colonic absorption values. This work sets the stage for improving colonic *in-vitro-in-vivo* correlations via physiologically based pharmacokinetic (PBPK) modeling, increasing understanding of colonic drug behavior.



Suspension-grown colonoids, A: apical-out, B: basal-out.

13 - In vitro model hydrogels to study diffusion properties of therapeutics

David Juriga[1] , *Per Hansson*[1]
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Abstract text
Subcutaneous (SC) injection stands as one of the most common administration routes of various therapeutics, including the COVID-19 vaccine or proteins and therapeutical peptides (TP). However, the significance of SC injection is rapidly growing, primarily due to the increasing number of newly developed therapeutical peptides in cancer therapy. The lack of knowledge regarding the diffusion and aggregation behavior of injected drugs indicates the importance of the development of *in vivo* or *in vitro* models.

In vivo animal models have several disadvantages, such as differences in chemical and physical structures compared to human adipose tissue. Conversely, natural-based polymeric systems such as hydrogels can mimic the environmental conditions of different tissues, therefore they are widely researched in medicine and pharmacy. Thanks to the advancements in microscopic and scattering techniques, we can have a better understanding of the interactions between drug molecules and the polymer matrix. This underscores the importance of developing new hydrogels based on polymers derived from adipose tissue.

The objective of this project is to create hydrogel-based model systems based on natural polymers and investigate the adsorption rate, diffusion, and bioavailability of different TPs. Within the scope of this project, several *in vitro* methods were developed to monitor the relationship between the aforementioned properties and the physico-chemical parameters, such as charge, size, and interaction with the polymer matrix, of the TP. To follow the diffusion of TPs fluorescence labeling and fluorescence recovery after photobleaching (FRAP) microscopy were used while the the mechanical and swelling properties of the hydrogels were characterized by rheometry and microfluidics-based methods. In the future, we plan to perform *in vivo* experiments with radioisotope-labeled TPs and uncover the correlation between the *in vivo* and the *in vitro* results. These findings can aid in formulating different TPs and designing new ones for the future.

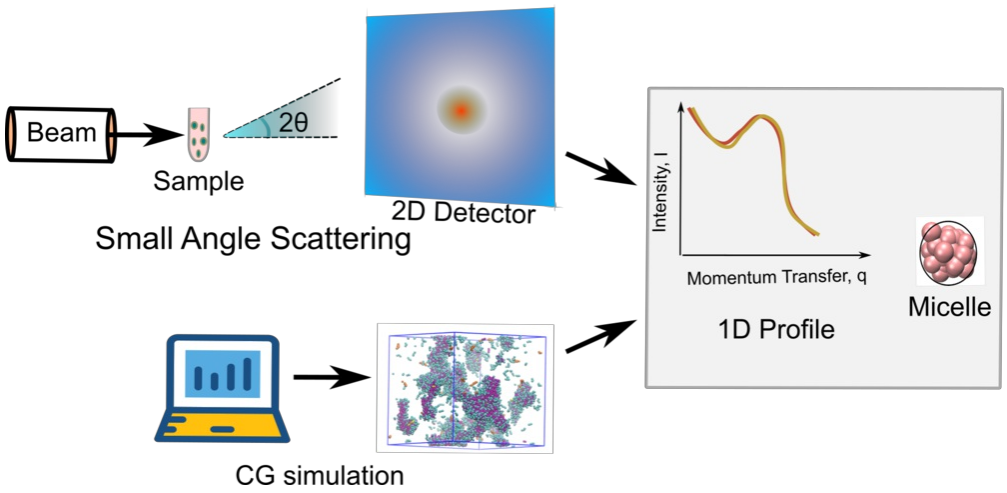
14 - Influence of intestinal colloidal structures and self-assembly on lipid-based formulations for enhancing peptide drug bioavailability

Shahina Akter[1] , *Per Larsson*[1]
[1] Uppsala University

Abstract text
Therapeutics based on peptides show promise in the treatment of conditions such as diabetes, cancer, and irritable bowel syndrome. Their big size and hydrophilic nature, however, make it difficult to achieve the best oral bioavailability because of the intestinal epithelium's low permeability. To improve bioavailability, different methods have been looked into, such as endocytic routes, microneedles, co-releasing transient "permeability enhancer" fatty acid-based molecules, and combining PE molecules with lipid-based excipients in pharmaceutical dosage forms.

The formation of colloidal and solubilizing structures by components of fatty acid formulation and other intestinal fluids occurs in the small intestine, which is integral to the process of drugs absorption. Due to the structural changes brought about by dilution, digestion, and absorption, the intestinal medium and formulation ingredients interact in a way that is rather intricate. The robustness of illness treatment is hampered by the unpredictability linked to lipid-based permeability enhanced administration.

To address these challenges, a comprehensive approach using small-angle X-ray and neutron scattering, and molecular dynamics simulations is proposed. This integrated framework aims to provide a detailed understanding of colloidal structures formed by the permeation enhancers, as well as taurocholate, and phospholipids, the major components of intestinal fluids. This knowledge will enable the tailored design of oral drug delivery systems for peptide drugs, addressing individual variabilities and increasing bioavailability.



15 - A new tool for the BioSAXS toolbox, AF4-UV-SAXS

Hans Bolinsson[1] , *Christopher Söderberg*[2], *Fátima Herranz-Trillo*[3], *Marie Wahlgren*[1], *Lars Nilsson*[1]

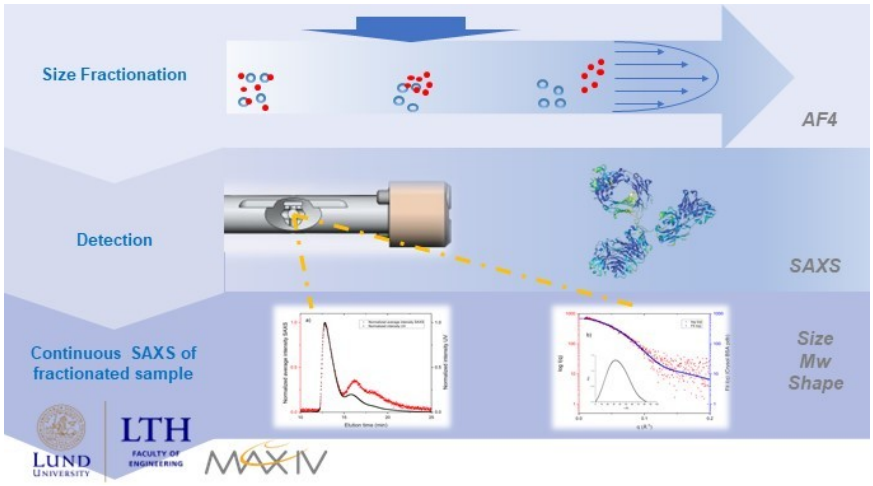
[1] Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden.

[2] RISE Research Institutes of Sweden, Division Bioeconomy and Health, Chemical Process and Pharmaceutical Development, Lund, Sweden.

[3] CoSAXS Beamline, MAX IV Laboratory, Lund, Sweden.

Abstract text

We demonstrate the coupling of synchrotron SAXS to AF4 for protein characterization. To the best of our knowledge this is the first time AF4 is successfully coupled to a synchrotron for on-line measurements on proteins. This coupling has potentially high impact, as it opens the possibility to characterize individual constituents of sensitive and/or complex samples, not suited for separation using other techniques, and for low electron density samples where high X-ray flux is required, e.g. biomolecules and biologics. AF4 fractionates complex samples in native or close to native environment, with low shear forces and system surface area. Many orders of magnitude in size can be fractionated in one measurement, without having to reconfigure the experimental setup. We report AF4 fractionations with correlated UV and statistically adequate SAXS data of Bovine Serum Albumin and monoclonal Antibody and demonstrate how SAXS results from size separated populations can be utilized for characterization of individual constituents of a complex sample assembly."



16 - Investigation of self-assembled aggregates of a lipidated glucagon-like peptide analogue in aqueous solutions using small-angle X-ray scattering

Ellen Brunzell[1] , *Kalle Sigfridsson*[2], *Magnus Bergström*[1]

[1] Department of Medicinal Chemistry, Pharmaceutical Physical Chemistry, Uppsala University, 751 23 Uppsala, Sweden.

[2] Advanced Drug Delivery, Pharmaceutical Science, R&D, AstraZeneca, 431 83 Gothenburg, Sweden.

Abstract text

Background: Aggregation of peptides can have a number of consequences; it can cause immunogenic reactions in vivo, it is linked to degenerative diseases, and it can reduce the intended effect of a peptide drug product, but it can also be utilised in extended-release formulations.[1–4] In this study, MEDI7219 is used as a model peptide to investigate the aggregation behaviour of an amphiphilic peptide. MEDI7219 is based on the sequence of glucagon-like peptide (GLP-1) and has been used as a model substance to study oral absorption of GLP-1 analogues.[5]

Methods: The self-assembled particles of 1-50 mg/ml MEDI7219 in solution were analysed with small-angle X-ray scattering (SAXS). The solvents used were water, 150 mM sodium chloride (NaCl) and sodium thiocyanate (NaSCN), phosphate buffer pH 7.4, phosphate buffer pH 7.4 with 200 mM sorbitol, and acetate buffer pH 5.5, in order to study the influence of added salt, pH, and buffer agents.

Results: Small-angle X-ray scattering (SAXS) data suggest that MEDI7219 assembles into small aggregates with a core-shell structure.

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17 - The impact of co-administration of solubilizing and amorphous formulations – An in vitro and in vivo evaluation

Caroline Fronczak Alvebratt[1] , *Filip Karlén[1], Michelle Åhlén[2], Khadijah Edueng[1], Ilse Dubbelboer[3], Christel A. S. Bergström[1, 4]*

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[2] Division of Nanotechnology and Functional Materials, Department of Engineering Sciences, Uppsala University, Sweden

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Abstract text

A range of enabling formulations like lipid-based formulations (LBFs) and amorphous solid dispersions (ASDs) have been developed to improve drug uptake from orally administered poorly water-soluble drugs. However, performance of different types of formulations are seldomly evaluated in the same in vitro assays, making head-to-head comparisons challenging.[2] In 2019, a new digestion-absorption device (ENA) was introduced, which showed good *in vitro-in vivo* correlation (IVIVC) for LBFs in pigs.[3] Due to the experimental setup of the ENA, it can be used for other enabling formulations as well. As the formulation strategies have different strengths and limitations, it would be of interest to investigate if co-administration could be applied as a strategy to improve the *in vivo* performance. The aim of this study was to (1) investigate the *in vitro* performance of one LBF, one ASD and the combination of the two in the ENA, and (2) explore the possible relationship between the ENA assay and the plasma concentration in rats. Felodipine was used as model compound and formulated in an ASD and an LBF. A blank LBF was also prepared for coadministration with the ASD. The *in vitro* performance of the formulations was evaluated in the ENA assay, sampling from both donor and receiver side of a semi-permeable membrane. The *in vivo* performance was evaluated in rats, where the formulations were administered in gelatin capsules. Both formulations, separately and combined, could be evaluated with the ENA. The ASD showed the lowest concentration in the donor chamber of the ENA *in vitro*, and a pH dependent solubility was observed. The apparent solubility was improved ~45-fold when combined with the LBF. The beneficial effect of the LBF_{Blank} on the performance of the ASD were also seen *in vivo*. Low bioavailability of felodipine formulated an ASD can be improved by coadministration of blank LBF.

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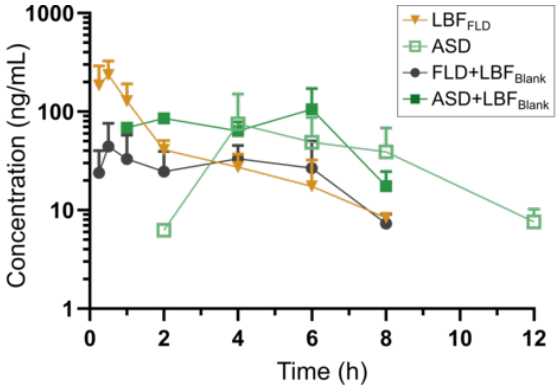

Plasma concentration in rats (mean±SD) after oral administration of capsules with different enabling formulations, separate and combined. LBF_{FLD}: Felodipine-loaded lipid-based formulation, ASD: Amorphous solid dispersion, FLD: Crystalline felodipine, LBF_{Blank}: Blank, drug-free LBF.

18 - Design of antioxidant nanoformulations for glaucoma prevention

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[1] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, SE-1 71 65, Sweden

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Abstract text

Glaucoma is the leading cause of irreversible blindness worldwide. Current therapies have been proven insufficient primarily due to poor drug bioavailability. Recently, nanoparticles have been used as effective carriers for drug delivery in the eye, because of their high surface-to-volume ratio enabling high drug loading values (Tsikourkitoudi *et al.*, 2020) and enhanced bioavailability.

Taking into consideration the unique properties of nanoparticles for eye drug delivery, here, we propose a novel drug delivery system consisting of biocompatible nanoparticles and small molecular drugs that promote nicotinamide adenine dinucleotide (NAD) production. NAD is an ideal target for glaucoma treatment associated with neuroprotection in glaucoma (Adams *et al.*, 2019). We synthesize silica (SiO_2) nanoparticles by flame spray pyrolysis (FSP) that is a highly reproducible and inherently scalable technique in which a liquid precursor is ignited and combusted. The nanoparticles are formed in the flame by nucleation, surface growth, coagulation, and coalescence and collected on a glass fibre filter. As-synthesized SiO_2 nanoparticles (specific surface area 216m^2/g, primary particle size 10nm) are first functionalized by 3-aminopropyl-triethoxysilane and then natural antioxidant molecules (gallic acid (GA) and epigallocatechin gallate (EGCG)) are covalently grafted on their surface with high loading values (90mg GA/$g_{particle}$ and 150mg EGCG/ $g_{particle}$).

The NAD-generating capacity of the nanoformulations is evaluated *in vitro* by luminometry assay on cortical neurons in suspension after 2h incubation. GA and EGCG-loaded SiO_2 nanoparticles induce a higher fold change of NAD compared to SiO_2 nanoparticles at equivalent particle concentrations. Our results imply that high drug loading delivery systems based on flame-made nanocarriers can improve therapeutic efficiency by delivering high concentration of drugs at the diseased sites. As the proposed drug delivery systems consist of biocompatible and natural compounds, the translation of the proposed formulations for glaucoma prevention, even in societies that lack adequate ophthalmic care, will be promoted.

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19 - Antimicrobial flame-made nanosilver particles supported on nanostructured silica against MRSA wound infections

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Abstract text
The global rise in antibiotic-resistant bacteria, primarily fueled by the overuse and misuse of antibiotics, presents a pressing public health concern, necessitating innovative approaches to combat infections. Silver nanoparticles (Ag NPs), with their distinctive physicochemical properties, offer a promising solution [1]. Additionally, the incorporation of silica (SiO_2) into composite Ag/SiO_2 nanoparticles enhances stability, prevents agglomeration, increases surface area, and augments their antibacterial properties [2].

In this study, Ag/SiO_2 nanoparticles were produced by flame spray pyrolysis (FSP), a highly scalable and reproducible aerosol-based synthesis method. Variations in Ag content (0%, 20%, 40%, and 60% wt) were achieved while maintaining a constant total metal concentration. Comprehensive characterization included specific surface area (SSA) measurement via N_2 adsorption, crystallite size determination using X-ray diffraction patterns, and structural analysis through TEM examination. The release of Ag^+ ions at various incubation timepoints was also quantified.

Antibacterial efficacy against Methicillin-resistant Staphylococcus aureus (MRSA) was evaluated in vitro via colony-forming units (CFUs) and spot plating, covering NP concentrations ranging from 12.5 to 100 µg/ml. To assess practical application in physiologically relevant contexts, ex vivo testing was conducted by applying varying nanoparticle concentrations to MRSA-infected porcine skin wounds.

Ag/SiO_2 nanoparticles demonstrated a notable reduction in CFUs/ml compared to SiO_2, positioning them as promising nanoantibiotic agents. The study reports a dose-dependent antibacterial efficiency of Ag/SiO_2 nanoparticles, with enhanced antibacterial efficacy observed in nanoparticles with higher Ag content. Remarkably, Ag/SiO_2 nanoparticles displayed antibiotic effects comparable to, and in some cases, surpassing those of vancomycin—an established MRSA antibiotic.

Overall, this study underscores the heightened potential of nanoparticle-mediated therapeutic strategies utilizing inorganic nanoparticles in the fight against antimicrobial infections. These findings hold promise for addressing the critical issue of antibiotic resistance, both in controlled laboratory settings and within physiologically relevant conditions.

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20 - Extracting mechanistic information from the unloading stage of tableting

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Abstract text
The compressibility and compactibility of powders are well-established in pharmaceutical research[1], however, there is a lack of insight into the relevance of processes at the particle level. Research efforts have primarily concentrated on the compression stage, driven by significant changes observed during this phase. However, the elastic recovery and bond breakage can be directly related to damage that occurs primarily during unloading[2].

This project aims to provide a mechanistic understanding of the type and extent of processes that are at play during unloading and how these processes are reflected in the unloading curve. One objective is to establish relationships between data obtained from analytical powder compression throughout the loading–unloading cycle and the independent determination of mechanical and structural characteristics of the resulting agglomerates.

Experimental work involved three different powders microcrystalline cellulose (Pharmacel 102), sieved lactose monohydrate (Pharmatose 125M), and Ibuprofen. The elastic recovery ($ER_{in\text{-}die}$) and elastic modulus (E_M) were calculated from the unloading data, revealing an upward trend in both with increasing applied pressure. Pharmacel 102 exhibited higher elastic recovery and lower elastic modulus, indicating greater elasticity. Ibuprofen and Pharmatose 125M displayed similar trends.

To delve into the observed behavior, reloading experiments were performed. For Ibuprofen, both unloading curves initially displayed viscoelastic behavior, transitioning to elastic behavior, and curving again during unloading (Figure 1). Pharmacel 102 exhibited similar patterns but with steeper curving after 50 MPa, suggesting viscoelasticity. Pharmatose 125M showed distinct variations between curves.

Additional experiments, included variations in lubricant concentration and punch speed. Lubricant concentration did not significantly affect the curves. However, decreasing upper punch speed reduced the distinction between curves, particularly at 1 mm/min.

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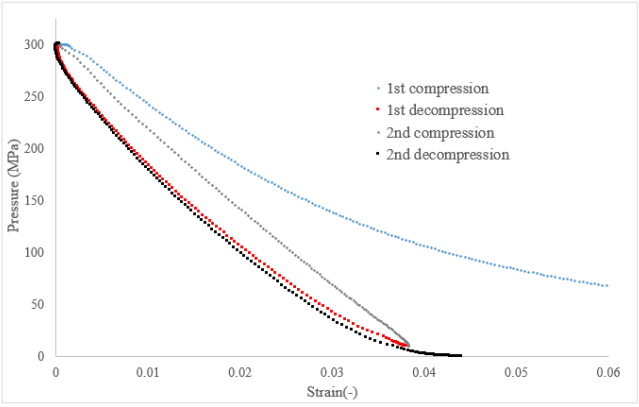

Figure 1 : Reloading profile for Ibuprofen

21 - Ethylcellulose oleogel as the potential controlled release delivery system

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Abstract text
In this work we investigate the potential of ethylcellulose (EC) oleogels as a controlled release system for probiotics. Oleogels are gels in which the continuous liquid phase is oil. The EC oleogels first investigated were quite firm when the concentration was higher than 10%. We have shown that these EC oleogel can retard lipolysis substantially (low-oil-release). As an example, using 10% EC cp300 (high viscosity) in the oleogel resulted in less than 40% completion of lipolysis after 2 hours. Thus, these EC oleogels have the potential to be a controlled release system that targets the later part of the small intestine and colon. This is interesting for probiotics as this might increase the survivability of the bacteria in the GI tract, where bile salts are a major challenge. However, the high retarding effect from EC oleogels also means a too-low release of the probiotics. Therefore, we have continued to investigate how to control the release pattern of these oleogels, for example, by investigating different molecular weights of the EC used and the concentration of EC in the oleogels. Furthermore, we have shown that the bacteria have high survivability both in EC oleogel formulations and under gastric conditions. SAXS data shows there are long-range and short-range structures in the oleogels, which can be related to the rheological properties of the oleogels. In conclusion, with a better understanding of EC oleogel structure, EC oleogels might be a promising formulation strategy for probiotics.

22 - Rheological and in Vitro Evaluation of Xeno-Free Mesoporous Silica Nanocomposite Biomaterial Inks for 3D Printing

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Abstract text

Developing xeno-free nanocomposite biomaterials suitable for hydrogel-based three-dimensional (3D) printing is in high demand to expand hydrogel use in biomedical applications. Previously developed binary wood-biopolymer system consisting of a photo(bio)polymer of methacrylated galactoglucomannan (GGMMA) and TEMPO-oxidized cellulose nanofibers (T-CNF) has shown high performance as biomaterial ink, yet does not suffice to provide protection and controlled release of biologicals alone. Nanocomposite formulation by incorporating nanoparticles into this natural polymer-based ink is a promising yet challenging alternative due to hydrogel stability being sensitive to any charged surface. Mesoporous silica nanoparticles (MSNs) are well-established nanocarriers for delivering different biologicals with high efficiency, and their surface can be modified to formulate nanocomposite biomaterials. In this study, we fabricated MSNs with different surface modifications resulting in a net surface charge ranging from highly negative to highly positive to develop printable mesoporous nanocomposite biomaterial inks. We utilized rheology as a comprehensive tool to address the physicochemical interactions between the hydrogel matrix and different surface-charged MSNs. Furthermore, we have used FITC-BSA as a model protein and evaluated the in vitro protein release and its thermal stability after 3D printing. Our results showed negatively or neutral-charged MSNs are suitable to formulate FITC-BSA-loaded nanocomposite biomaterial inks. Depending on the particle's surface charge, FITC-BSA showed different release profiles and preserved its stability after release. In conclusion, the developed nanocomposite biomaterial inks are 3D printable with the advantage of delivering various biomacromolecules in tissue engineering and regenerative medicine.

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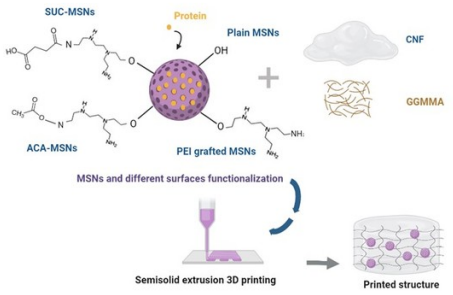

23 - Identification of lipid-based formulations with best in vivo performance making use of the enabling absorption (ENA) device

Lingxiao Li[1] , *Christel Bergström*[1]
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Abstract text
Lipid-based formulations (LBFs) has been developed for decades as one formulation strategy to solve the problem of low aqueous solubility of active pharmaceutical ingredients (API). Poorly water-soluble, lipophilic compounds hold higher solubility in lipids, making more drug molecules available for absorption, thus increasing the bioavailability. In vitro lipolysis is the most common method to study the dissolution performance of an LBF, as the experiment mimics the digestion process in the gut and at the same time provides information of drug solubilization and absorption in vivo. However, data from lipolysis experiment may fail to predict the in vivo ranking order of LBFs and one of the reasons is the lack of an absorptive sink. The Enabling Absorption (ENA) device was developed to address this issue, composed of a donor and a receiver compartment, separated by an absorptive membrane composed of Caco-2 cells or an artificial membrane (lecithin-in-dodecane; LiDo). In this study, three danazol LBFs (F1, F2 and F4) containing different proportion of a long-chain lipid soybean&maisine and surfactant Cremophor EL from which in vivo data in dogs were published by Cuiné et al. were prepared and tested in ENA device. Samples were taken from both the donor and receiver to investigate the drug release, digestion, distribution and permeation. HPLC-UV was used for danazol quantification. The three LBFs showed different drug distribution profile to oil, aqueous and solid (precipitate) phases identified in the donor chamber as a result of being digested by immobilized lipase. Interestingly, the dose-adjusted flux provided the same ranking order of three LBFs as the result from beagle dogs, with a good correlation (r^2 = 0.94) when plotting the area under the curve (AUC) data. The results suggested that ENA device is a valuable tool to study the in vitro digestion-permeation of LBF, providing useful prediction for in vivo.

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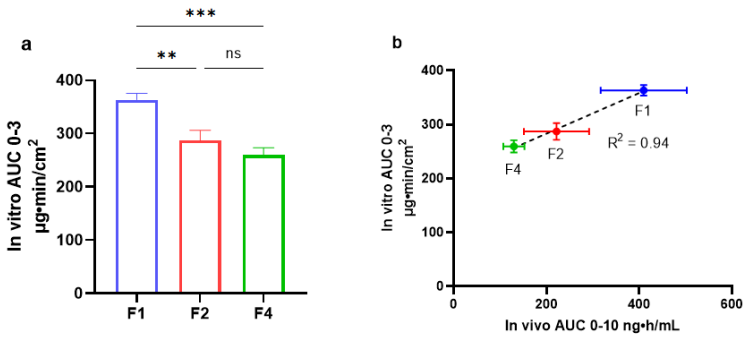

Figure 1. Dose-adjusted AUC of danazol permeation: a. AUC of danazol flux into the ENA receiver, F1 (blue), F2 (green) and F4 (red); b. IVIVC of in vivo danazol plasma exposure (from beagle dogs) and in vitro danazol trasnfer across the membrane to the receiver in ENA. Values are presented as means ± SD (n=3).

24 - Effect of drug load on the aerosolization propensity of binary adhesive mixtures for inhalation

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Abstract text

The aim of this study was to investigate how the aerosolization propensity of binary adhesive mixtures was affected by the drug load and whether these findings could be linked to different blend states. The study involved using two different lactose carriers, each with varying size and morphology, along with budesonide (API). In vitro aerosolization studies were conducted at four different pressure drops ranging from 0.5 to 4 kPa, utilizing a Next Generation Impactor. Several dispersion parameters were derived from the relationships between the amount of dispersed API and pressure drop. These parameters include:

1. FPF_{max}, $MMAD_{min}$: These parameters represent the maximum fraction of fine particles and the minimum mass median aerodynamic diameter of the fine particles that can be aerosolized from the mixture under the given conditions.
2. PD sensitivity: This parameter measures how sensitive the dispersion of the mixture is to changes in pressure drop.
3. k_d: This parameter indicates how quickly the drug particles disperse at varying pressure drops.

The evolution of the parameters with drug load was complex, especially at low drug loads. While similar patterns were observed for both carriers, the range of drug load that could be used varied significantly due to differences in the size and morphology of the carriers. These carrier properties not only influenced the drug loading capacity but also affected the spatial distribution of the API within the mixture, which in turn impacted their aerosolization propensity. The evolution of drug dispersion with drug load could thus be linked to different configurations of the lactose carrier and budesonide in the blends, denoted blend states.

The study suggests that the concept of blend states can provide a better understanding of the complex dispersion process of adhesive blends. This information could be valuable in optimizing the formulation of inhalable drugs ensuring effective aerosolization and drug delivery.

25 - Hybrid Phenol-Functionalized Nanoparticles for Reactive Oxygen Species Scavenging and Alleviation of Inflammation-Related Diseases

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Abstract text

Reactive oxidative stress (ROS) is linked to many acute and chronic inflammatory diseases, such as arthritis, inflammatory bowel disease, and diabetes. Yet, few clinical treatments are available to address this issue. Natural-derived polyphenols are known for their excellent antioxidant properties, which can help prevent and alleviate various ROS-related diseases. Unfortunately, the water solubility of natural-derived polyphenols is extremely low, making them almost impossible to be absorbed in the body, reducing their potential therapeutic benefits. Hence, developing nanomaterials with good ROS scavenging ability and biocompatibility is a promising way to treat ROS-related inflammation. Here, we present hybrid-phenol nanoparticles (HPNPs) for treating ROS, synthesized by one-step polymerization or by functionalizing the organic molecules onto inorganic amorphous SiO_2 nanoparticles. We selected representatives of the most common natural-derived polyphenols, such as stilbenoids (curcumin and resveratrol) and flavonoids (quercetin and gallic acid) for synthesizing HPNPs. After their synthesis, the HPNPs improved their aqueous solubility and long-term stability. Moreover, FTIR and Raman analyses proved the presence of the antioxidant OH-groups on the surface of the NPs. DPPH radical scavenging assay confirmed the ROS scavenging activity of HPNPs when compared to free polyphenols. Finally, the HPNP antioxidant effect was further validated *in vitro* after rescuing human embryonic kidney (HEK) cells from H_2O_2 oxidation and hypoxia. These findings lay the groundwork for using natural-derived polyphenol nanoparticles for ROS scavenging and demonstrate their potential use in clinical applications.

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26 - Fabrication of stimuli-responsive nanogel for drug delivery applications

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Abstract text

Introduction:

Nanogels are cross-linked polymer-based hydrogel nanoparticles, and due to their superior properties including high drug loading capacity, low toxicity and stimuli responsiveness, they are considered as next-generation drug delivery systems[1].

Aim:

To synthesize a stimuli-responsive nanogel as a nanocarrier for the drug delivery.

Method:

Thermo-pH-responsive plasmonic nanogel (Ng) was synthesized by grafting poly (N-isopropyl acrylamide) (PNIPAM) to chitosan (CS) in the presence of chemical crosslinker to serve as a drug carrier agent[2]. We applied Fourier transform infrared spectroscopy (FTIR) to confirm successful synthesis of Ng based on the CS and PNIPAM. The developed nanogel formulation was thoroughly characterized by particle size distribution and zeta potential measurements in 4 different temperatures, Morphology and size measurements of nanogel were determined by transmission electron microscopy (TEM).

Results:

Nanogel was found to have a hydrodynamic size of approximately 167 nm in diameter. It is observed that the particle size decreased significantly in 37 ° C, which lead to release of drug in body temperature.

Conclusion:

In this work stimuli-responsive nanocarrier using synthetic and natural polymer for drug delivery applications were synthesized [3].

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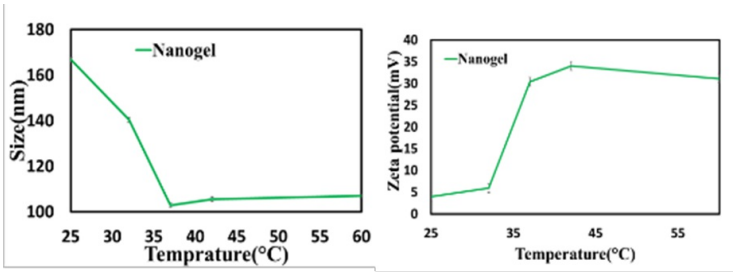
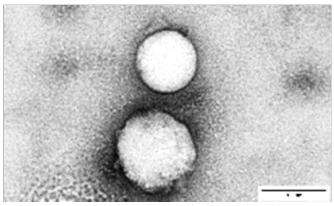

Nanogel
Size(nm)
180
160
140
120
100
25 30 35 40 45 50 55 60
Temprature(°C)
Nanogel
Zeta potential(mV)
40
35
30
25
20
15
10
5
0
25 35 45 55
Temperature(°C)



27 - pH-Responsive Nanoparticles for Precision Therapy of Rheumatoid Arthritis

Gizem Erensoy[1] , *Luca Dirk Menges*[1, 2], *Endri Bardhi*[1], *Loise Råberg*[1], *Ula von Mentzer*[1], *Alexandra Stubelius*[1]

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[2] Department of Virology, Hiedelberg University Hospital, Germany

Abstract text

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that causes cartilage and bone destruction. Despite the improvement in clinical outcomes, a third of patients still fail to respond [1]. To achieve early and rapid disease remission, systemic glucocorticoids are used as adjunctive therapies, however, the risk of side effects may not outweigh the treatment benefits. In addition, RA's high pathogenic heterogeneity poses further issues hindering optimized treatment strategies [2]. The goal of this study was to develop more advanced and accurate drug-delivery systems. Our approach is based on the strategy that smart materials can react and release drug based on the pathogenic inflammatory microenvironment. The dynamic microenvironment of inflammation requires highly sensitive systems that can rapidly both turn ON, and crucially, also be turned OFF. This study investigates the ability of formulated pH-responsive acetalated dextran (AcDex) nanoparticles to turn ON/OFF/ON. We demonstrate a sensitive and dynamic release system by repeatedly varying the pH from 6 to 7.4 (Fig 1). A dose-dependent release of model cargo was observed with macrophages stimulated with our without LPS. Validating this system with patient material is important to establish the therapeutic advantage of this flare-responsive drug delivery approach.

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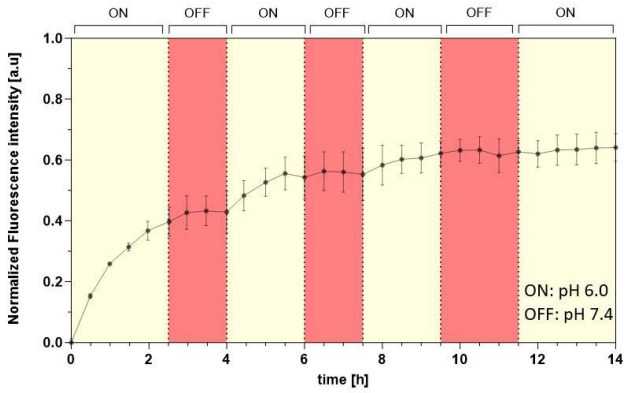

Figure 1: ON/OFF/ON release system for AcDex nanoparticles.

28 - Personalizing Oral Delivery of Nanoformed Piroxicam by Semi-Solid Extrusion 3D Printing

Rathna Mathiyalagan[1] , *Erica Sjöholm[1], Sajana Manandhar[2], Satu Lakio[2], Jessica M. Rosenholm[1], Martti Kaasalainen[2], Xiaoju Wang[1], Niklas Sandler[1, 2]*

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Abstract text

Poorly water-soluble drugs fail to reach the manufacturing process due to low aqueous solubility, leading to low bioavailability and efficacy. Different particle engineering techniques have been used recently to improve the solubility of drugs by reducing the size of drug particles to solve this issue. Controlled Expansion of Supercritical Solution (CESS®) based on supercritical carbon dioxide ($scCO_2$) was the nanoforming technology that produces pure dry drug nanocrystals without requiring excipients. As the particle size of nanoformed material decreases with the increased surface area. Thus resulting in improved solubility and a faster dissolution rate. Piroxicam (PRX), a poorly water-soluble drug with good permeability, was nanosized with CESS®. This study explored CESS® produced nanosized piroxicam (nanoPRX) as a model drug to investigate the stability and to achieve personalized oral dosage forms with different sizes utilizing semi-solid extrusion (SSE) 3D printing technology. Tylopur-605, a hydroxypropyl methylcellulose (HPMC) based polymer, was used to stabilize the NanoPRX in the aqueous solution. The prepared suspension was combined with two different HPMC and hydroxypropyl cellulose (HPC) polymer solutions and mixed with a microfluidic pump. Two suitable printing inks were prepared, and SSE 3D printed to produce personalized thin oral films.

The CESS® produced nanoPRX was successfully stabilized and 3D printed with SSE. The different sizes of nanoPRX oral dosage forms with two different HPMC and HPC-based printing inks were obtained with a high correlation of R^2=0.9981 and R^2=0.9988 between the designed size and obtained drug amount. This study investigated that the CESS® produced nanoPRX was successfully suspended, dispersed, and stabilized in the aqueous base solutions. The HPMC polymer successfully stabilized the nanoPRX in the suspension, and suitable printing inks were produced by mixing with a microfluidic pump. The accurate nanoPRX containing oral thin film dosage forms with great correlations were produced by the SSE 3D printing technique.

29 - Enhanced Spray-dried Formulations of Inhaled Biotherapeutic Drugs

Mayura Talwelkar Shimpi[1] , *Göran Frenning*[1]
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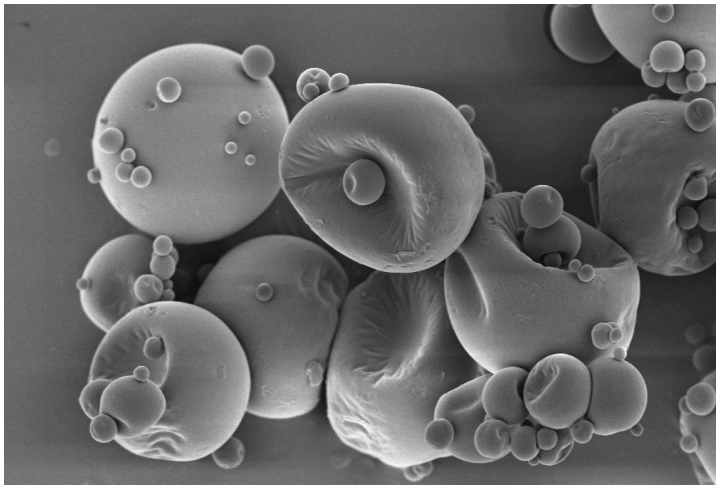
Abstract text
Dry powder inhalers (DPIs) are a class of inhaled drugs which shows many advantages over other inhaled drug products such as increased stability of formulations due to the dry state, portable nature, ease to operate devices and propellent free. In this work, trehalose and pullulan are explored as a potential excipient system and their effect on the morphology, physical properties and stability of spray dried DP formulations of biologics and small drug molecules is assessed. Trehalose has been explored as an excipient in DP formulations of proteins and shows excellent a potential to form a stable DP formulation. However, trehalose possesses a low glass transition temperature (Tg) which results in hygroscopic powder formulations with low shelf life. Pullulan, a polysaccharide with high Tg, is well explored in various drug delivery systems, but its application in DP formulations is still very limited. In the present work, the effect of pullulan on the spray dried pullulan-trehalose powders was analysed using DSC for moisture content and glass transition temperature. The DSC analysis shows that the Tg of trehalose shifted to higher temperatures in the presence of pullulan and it is also dependent on the percentage of the pullulan in the blend. The particle size and morphology were studied using a Malvern particle size analyzer 3000 and SEM, respectively. The preliminary results of the study show that the percentage of the pullulan in the blend significantly affects the particle size and the morphology of the product. Higher pullulan ratio resulted in particles with corrugated surfaces compared to the spherical particles obtained for higher trehalose content. Thus, trehalose-pullulan mixtures show promising results as a potential excipient system for spray dried DP formulations of biologics for better stability and shelf life.



30 - Development and assessment of multi-component materials for 3D-printed personalized and customized drug delivery - 3D Cure

Ezgi Özliseli[1] , *Ellen Sundström[2], Usama Jamshaid[1], Rathna Mathiyalagan[1], Alaa Mahran[1], Sofia Lisina[1], Mirja Palo[1], Kuldeep Bansal[1], Xiaoju Wang[1, 2], Jessica Rosenholm[1], Chunlin Xu[2],* ***Tapani Viitala***[1]

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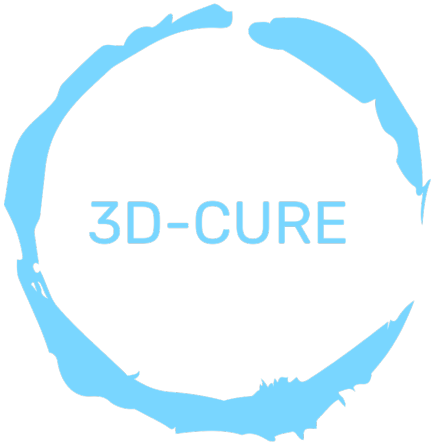
Abstract text

3D-printing (3DP) holds the potential to become a disruptive technology in the pharmaceutical industry. 3DP facilitates the use of multicomponent materials and complex geometries, which are difficult to manufacture with conventional methods, and which are potential both for better one-fits-for-all products (e.g., better efficacy, less side-effects), and for personalized medication. Despite the promising prospects, the pharmaceutical industry has identified several needs and challenges related to 3DP, such as lack of medical grade materials suitable for a wide variety of applications, lack of clear regulations for 3D-printed drug and combination products, lack of real-time/online monitoring technologies for QA and lack of an in depth understanding of the interplay between material properties and process parameters and how these affects the quality target product profiles (QTPPs) of 3D-printed products for personalized and customized drug delivery.

The 3D-CURE project brings together four different research organizations and several companies. Turku University of Applied Sciences, Åbo Akademi, University of Turku and Lappeenranta-Lahti University of Technology together with the companies Bayer, Brinter, Gasera, CH-Bioforce, UPM Biomedicals, Rokote Laboratories, DelSiTech and EDR & Medeso form a consortium to develop a wide range of complementary competencies. The consortium covers the entire value chain from raw materials to design, manufacturing and quality management of new pharmaceuticals and medical technology products with multiple components as well as 3D printing of these. The project hopes to generate new materials and knowledge about the suitability of 3D printing for pharmaceutical manufacturing as well as explore the possibilities for real-time monitoring of the 3D printing process. The special focus for Åbo Akademi in the project is to develop different multicomponent biopolymer-based materials and to assess and improve the knowledge about their properties, and how these and the 3DP process parameters are linked to the QTPPs of 3DP constructs for immediate and tunable drug delivery.



31 - Lipid nanocarrier loaded dissolving microneedles for local treatment of vaginal fungal infections

Paarkavi Udayakumar[1] , *Cristhian Salas Cotaquispe[1], Georgios Sotiriou[2], Natasa Skalko-Basnet[3], Juan Du[2], Alexandra Teleki[1]*

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Abstract text

Introduction

More than 75% of women are susceptible to vulvovaginal candidiasis (VVC), and the majority show a recurrent form of the infection. There is a need for formulations with high mucus penetration and a sustained drug release to improve current treatment options for VVC (1). Here, we develop dissolving microneedles (MNs) for vaginal delivery of clotrimazole.

Methods

Clotrimazole was formulated into two different types of lipid nanocarriers (LNCs) i) oil-in-water nanoemulsions (2) and ii) liposomes (3). The hydrodynamic diameter (d_{DLS}) of the LNCs was determined by dynamic light scattering. MNs of varying heights (600-1000 mm) were prepared using a silicone mold. LNCs were incorporated in the MN tips composed of water-soluble polyvinyl alcohol. Polymethyl methacrylate was used as an insoluble backing layer (4). The MNs were characterized by scanning electron microscopy and their penetration in *ex vivo* bovine vaginal tissue was measured with a texture analyzer at controlled insertion force. Drug release from the LNCs and MNs was studied in a Franz cell diffusion set-up and the anti-fungal efficacy was evaluated using clinical isolates of *C. albicans.*

Results

LNCs were incorporated in MN tips at various tip heights. Liposomes (d_{DLS}=170 nm, 90% entrapment efficiency) exhibited sustained drug release for six hours *in vitro*. A higher drug loading (50 mg/g) could be achieved in nanoemulsions (d_{DLS}=230 nm, 84% entrapment efficiency) compared to liposomes. d_{DLS} in both formulations was stable upon storage at RT for two weeks. MN tips rapidly dissolved upon insertion on bovine vaginal tissue and >50% reduction in needle height was observed. The LNC-loaded MNs effectively inhibited growth of *C. albicans.*

Conclusion

Clotrimazole-loaded LNCs were optimized for their size and drug recovery. The LNCs were formulated into dissolving MN tips to enable mucus penetration and sustained drug release for a prolonged period of time for local treatment of VVC.

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32 - Directing cellular responses in nanocomposite 3D matrix for tissue regeneration with nanoparticle-mediated hydrophobic drug delivery

Ezgi Özliseli[1] , *Sami Şanlıdağ*[2, 3, 4], *Behice Süren*[1], *Alaa Mahran*[1, 5], *Marjaana Parikainen*[2, 3, 4], *Cecilia Sahlgren*[2, 3, 4, 6, 7], *Jessica Rosenholm*[1]

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Abstract text

Hydrogels play an important role in tissue engineering, due to their distinct characteristics resembling native extracellular matrix.[1] However, they do not inherently offer sufficient stimuli to support tissue formation. While the incorporation of bioactive cues directly into hydrogels has been explored, limitations such as incompatibility with hydrophobic drugs, issues of burst/uncontrolled release, and rapid degradation of the bioactive molecules prevail.[2] Here, nanoparticle-hydrogel composite is formulated as a potential to combine the benefits of controlled delivery of bioactive cues and cellular support using Mesoporous silica nanoparticles (MSN). The surface functionalization feature of MSNs as constituents of the nanocomposite hydrogel platform was exploited for optimized homogeneity and cellular response to achieve intracellular drug delivery in 3D matrix. The results showed that all surface-modified MSNs were internalized by progenitor cells rapidly from the 3D matrix, moreover, MSNs modified with succinylation and acetylation, which have negative or neutral charge, respectively, showed superior cellular uptake and simultaneously allowed efficient local intracellular delivery of a hydrophobic model drug. Evaluation of the formulated nanocomposite hydrogel efficiency was performed furthermore using Notch signaling inhibitor (DAPT), and MSN-PEI-SUC-hydrogel nanocomposites were clearly able to downregulate the Notch signaling target genes in 3D and elevate the myogenesis markers. These findings present effective surface engineering strategies for designing nanocomposite hydrogels, and these systems can be utilized to develop smart biomaterials for tissue engineering.

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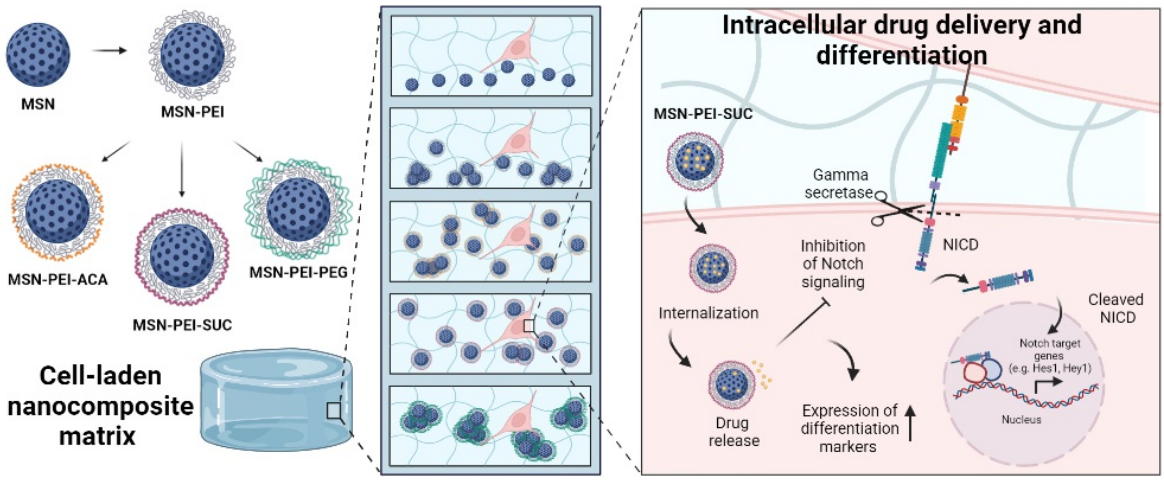
MSN
MSN-PEI
MSN-PEI-ACA
MSN-PEI-PEG
MSN-PEI-SUC
Cell-laden nanocomposite matrix
Intracellular drug delivery and differentiation
MSN-PEI-SUC
Gamma secretase
NICD
Inhibition of Notch signaling
Internalization
Cleaved NICD
Notch target genes (e.g. Hes1, Hey1)
Nucleus
Drug release
Expression of differentiation markers

33 - Development and optimization of iron oxide nanoparticles for dual T1/T2 magnetic resonance imaging

Qianying Chen[1] , *Shaquib Ansari*[1], *Alexandra Teleki*[1]
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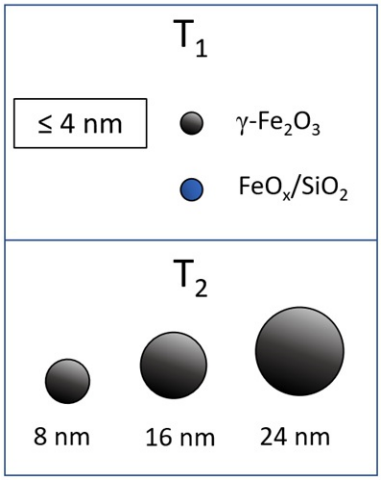
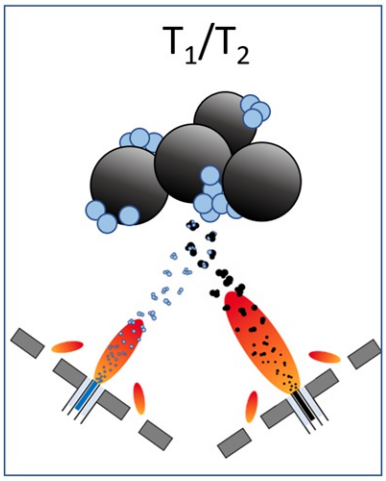
Abstract text
Magnetic resonance imaging (MRI) typically requires the use of contrast agents to discover disease lesions. T_1 and T_2 contrast agents are used to enhance the contrast between normal and diseased tissues in T_1- and T_2-weighted images, respectively. In the clinic, gadolinium-based contrast agents are used for T_1 contrast enhancement, especially of fatty tissue. However, they are associated with acute nephrotoxicity. T_2 contrast agents such as superparamagnetic iron oxide nanoparticles (SPIONs) are superior in imaging edema and inflammation. However, these two types of contrast agents have drawbacks leading to limited applications. T_1 contrast agents have decreasing efficiency at a high magnetic field. T_2 contrast agents disturb the magnetic field on surrounding normal tissues and these normal tissues will resemble diseased tissue by appearing dark (blooming effect). To overcome the limitations of separate contrast agents and improve diagnostic accuracy, dual T_1/T_2 contrast agents are developed. The T_1 efficiency of SPIONs can be improved by decreasing their particle size (≤ 4 nm). Thus, in the present work, nanoparticles with varying sizes (3, 8, 16, and 24 nm as determined by X-ray diffraction) and chemical composition (γ-Fe_2O_3 and FeO_x/SiO_2) were produced by flame spray pyrolysis (FSP). Pure γ-Fe_2O_3 nanoparticles were coated with citrate to improve aqueous dispersibility. The nanoparticles were characterized for their structural and magnetic properties and their MRI performance was assessed. The composite particles with 3 nm FeO_x supported on silica exhibited the highest T_1 relaxivity (r_1 = 2.3 s^{-1}(mM Fe /L)$^{-1}$). γ-Fe_2O_3 nanoparticles, 16 nm in crystallite size, displayed a high T_2 relaxivity (r_2 = 411 s^{-1}(mM Fe /L)$^{-1}$). Both nanoparticles showed visibly high contrast-amplifying effects in T_1- or T_2-weighted images. To utilize the efficiency of these two nanoparticles as dual T_1/T_2 contrast agents, a double-nozzle FSP will be used to combine the two nanoparticles into one modality.





34 - Lipid digestion and peptide stability of acylglycerol nanoemulsions

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Abstract text

To treat gastrointestinal diseases, the targeted delivery of therapeutic peptides with a high efficiency is desirable. Lipid-based formulations (LBFs) are successfully used as oral delivery systems for lipophilic drugs and biologics and can both protect the peptide from degradation as well as include transient permeation enhancers (TPEs) which can improve peptide uptake into the targeted cells. The degradation kinetics of the LBFs and thereby the simultaneous release of the incorporated drug and the TPEs is essential for successful drug delivery. Digestibility of LBFs is usually compared by analyzing the *in vitro* lipolysis, but the results from this method strongly depend on experimental conditions and formulation characteristics. Therefore, we introduced the intrinsic lipolysis rate (ILR) as a tool to compare lipid digestibility independent from the droplet size and interfacial structure. First, it was found that the amount of Polysorbate 80 used to stabilize tricaprylin nanoemulsions influenced the length of the lag phase and the ILR. The higher the amount of surfactant, the more the lipolysis of tricaprylin nanoemulsions was inhibited, i.e. the lower the ILR. Therefore, only lipolysis of emulsions with the same surfactant concentration can be compared. ILR of nanoemulsions with a constant and low surfactant content decreased with increasing acyl chain length, esterification and unsaturation. The ILR of the pure components can be used to accurately predict the ILR of component mixtures and pure components that cannot be experimentally determined as they do not form stable emulsions. Furthermore, by simply blending the model peptide drug octreotide (used for the treatment of acromegaly) with a prepared nanoemulsions, the presence of nanoemulsions stabilizes octreotide during lipid digestion. The ILR is a promising tool for systematic design of peptide-loaded LBFs with tailored digestion and thereby amount of *in situ* generated TPEs.

35 - Formulation of starch microspheres in aqueous two-phase systems: Exploring the microsphere formation process

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Abstract text

Starch microspheres can be prepared in emulsified aqueous two-phase systems (ATPS). The preparation of starch microspheres in ATPS offers several benefits, such as ATPS low interfacial tension, starch's ability to recrystallise at moderate temperature (through physical cross-linkage), as well as the absence of organic solvents and chemical cross-linkers. These benefits make the concept of starch microsphere preparation through ATPS promising for microencapsulation and delivery of sensitive cargo, such as biologics and live biotherapeutics. In order to exploit this system for future development, efforts have been made to characterise and better understand physical cross-linkage ATPS starch microsphere preparation. It has been shown that starch microsphere formation and product properties depend on several factors [1-4]. Even so, both microsphere formation and ATPS are complex, and the fundamental knowledge regarding these phenomena is still limited. Thus, a deeper understanding regarding these systems is needed.

In this project, we are exploring physical cross-linkage ATPS starch microsphere preparation and how different preparation parameters may be adapted. We are acquiring a better understating of starch-ATPS phase behaviour, as well as how to influence the starch crystallisation and microsphere formation process.

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36 - Subcutaneous delivery of antisense oligonucleotide beyond monthly - Biodegradable silica depots

Linda Wirman[1] , *Peter Gennemark[2, 3], Marceline Akieh-Pirkanniemi[2], Mika Koskinen[1], Nigel Davies[4], Marie Elebring[2], Anna Tivesten[5], Marie Strimfors[6], Asmaa McGowan[1], Mikko Hölttä[2], Magnus Söderberg[7], Veronica Berntsson[4], Daniela Balas[4], Lasse Leino[1],* ***Susanna ABRAHMSEN ALAMI***[8]

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Abstract text

Single-stranded antisense oligonucleotides (ASOs) are typically administered subcutaneously once weekly or monthly. Several technologies are available to provide sustained drug release via subcutaneous (SC) administration. ASOs have a high aqueous solubility and require relatively high doses which limits the options available substantially. In the present work, we show that an innovative biodegradable, non-porous silica-based matrix provides zero-order release in vivo (rats) for at least four weeks for compositions with ASO loads of up to about 100 mg/mL (0.5 mL injection) without sign of initial burst (Figure 1). This implies that administration beyond once monthly can be feasible providing potential to improve patient convenience, increase adherence and thereby for some diseases result in more optimal therapeutic outcomes. For higher drug loads substantial burst release was observed during the first week. The concentrations of unconjugated ASO levels in liver were found comparable to corresponding bolus doses. Additionally, infusion using minipump shows a higher liver exposure than SC bolus administration at the same dose level and, in addition, clear mRNA knockdown and circulating protein reduction comparable to SC bolus dosing, hence, suggesting productive liver uptake for a slow-release administration.

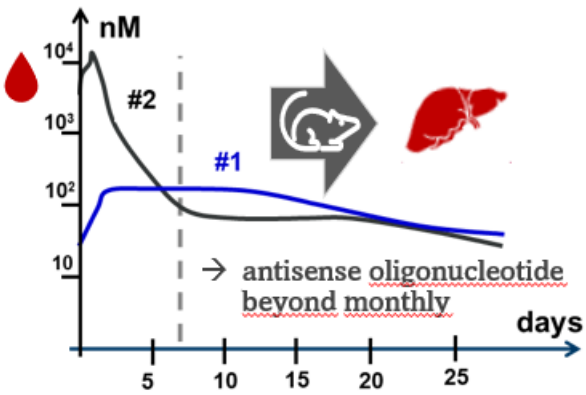

Figure 1
Rat blood plasma concentration (nM) after SC injection of two Silica Depot formulations of antisense oligonucleotide illustrating consecutive liver uptake.

#1~100 mg/mL
#2~150 mg/mL

37 - Targeting glial cells by Organic anion transporting polypeptide 1C1 (OATP1C1) -utilizing L-Thyroxine-derived prodrugs

Santosh Kumar Adla[1] , *Arun Kumar Tonduru*[1], *Seyed Hamed Maljaei*[1], *Landry Anamea*[1], *Janne Tampio*[1], *Adéla Králová*[1], *Aaro J. Jalkanen*[1], *Catarina Espada*[1], *Inês Falcato Santos*[1], *Ahmed B. Montaser*[1], *Jarkko Rautio*[1], *Thales Kronenberger*[1], *Antti Poso*[1], *Kristiina M. Huttunen*[1]

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Abstract text

OATP1C1 (Organic anion transporting polypeptide 1C1) is an organic anion-transporting polypeptide that belongs to the solute carrier family (SLCO1C1, also known as OATP14 or OATP-F or SLC21A14) and is a primary thyroid hormone transporter. It was characterized as a sodium-independent transporter which is originally localized to the brain and testes. In the human fetal brain, OATP1C1 is expressed in astrocytes and glial cells. It plays a crucial role in transporting thyroid hormones (TH), the pro-hormone thyroxine (T_4) and reverse triiodothyronine (rT_3) with high affinity.

In this study, we investigated the potential of utilizing OATP1C1 to improve delivery of anti-inflammatory drugs into glial cells. We designed and synthesized eight novel prodrugs by incorporating T_4 and 3,5-diiodo-L-tyrosine (DIT) as promoieties to selected anti-inflammatory drugs. The evaluation of prodrug uptake in OATP1C1-expressing human U-87MG glioma cells demonstrated higher accumulation of prodrugs with T_4 promoiety compared to those with DIT promoiety or the parent drugs themselves. *In silico* models of OATP1C1 suggested dynamic binding for the prodrugs, wherein the pose changes from vertical to horizontal. The predicted binding energies correlated with the transport profile of the prodrugs, with T_4 derivatives exhibiting higher binding energies when compared to the ones with DIT promoiety. Interestingly, the mouse brain transported prodrugs also showed utilization of oatp1a4/1a5/1a6 in mouse primary astrocytes, which was further supported by docking studies.

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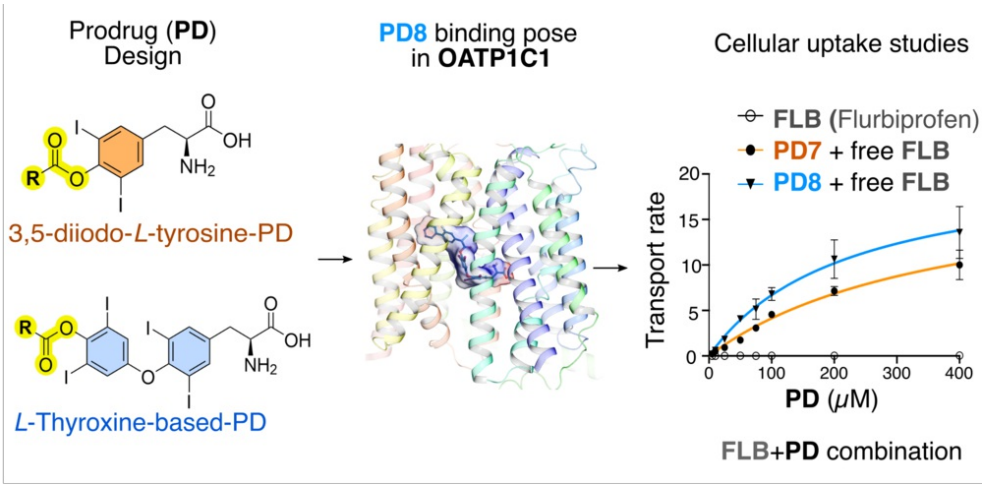

38 - Stable crystalline nanoparticles for oral delivery of antibiotics with less side effects.

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[3] Swedeliver

Abstract text

To rationally design and physically characterize oral antibiotic delivery systems, we focused on their stability towards enzymatic digestion in the gastrointestinal tract (GIT). An emerging class of advanced materials for drug delivery is non-lamellar liquid crystalline nanoparticles (LCNPs, e.g., hexosomes and cubosomes) that can be customized to target particular medical applications[1-2]. Despite their capability as carriers, their use has been limited due to the restricted range of building blocks in their production. In our research, we explored α-tocopherol (TCP) and farnesol (FAR) in phytantriol (PHY) -based LCNPs as potential systems for encapsulating vancomycin (VCM) and clarithromycin (CLM) to combat Methicillin-resistant Staphylococcus aureus (MRSA). We employed dynamic light scattering (DLS) to ascertain the size and charge of LCNPs and used small angle x-ray scattering (SAXS) to confirm their internal structure. Further assessments included drug loading experiments, in vitro digestion, and cargo release studies to determine capacity and stability. We found LCNPs ranging from 200 to 300 nm with a zeta potential of -15 to -25 mV that remained stable across temperatures from 10 °C to 50 °C. Encapsulation efficiency varied between 70-90% for VCM and 60-80% for CLM. These LCNPs resisted enzymatic digestion in the GIT, as proven by in vitro lipolysis assays, and effectively protective for Enterococcus and E.coli. In summary, LCNPs show promise as a potential oral delivery system for vancomycin and clarithromycin.

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39 - Determination of Solubility of Poorly-Water Soluble Drugs in Artificial Colonic Mucus

Mingjun Wu[1] , *Eva Karlsson*[2], *Christel Bergström*[1]

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Abstract text

Colonic mucus has gained in interest as a dissolution compartment in the large intestine due to the large fraction of bound water. Notably, the mucus layer contains more water than is freely mobile within the colonic lumen. Understanding the drug solubilisation in mucus is in favour of the refinement of *in silico* models for the prediction of drug dissolution and absorption in the colon. Here, we studied the solubility of drugs classified under Biopharmaceutics Classification System (BCS) II and IV in an artificial mucus solution. The mucus solution, designed to mimic the composition of porcine colonic mucus, was prepared with mucin, phosphatidylcholine, bovine serum albumin and cholesterol. Equilibrium solubility of sulfasalazine, febuxostat, hydrocortisone, prednisolone, fenofibrate, ticagrelor, and apixaban in the mucus solution (pH 7.0) and fasted-state simulated colonic fluid (FaSSCoF, pH 7.8) at 37ºC were assessed using the shake-flask method. Samples were taken at 24 h, 48 h and 72 h. Quantification of drug concentration was performed using either a plate reader or ultra-performance liquid chromatography (UPLC). Sulfasalazine and febuxostat exhibited solubility values in FaSSCoF that were 1.2 to 1.4 times higher than those observed in the mucus solution, while there was an opposite trend for the other five drugs. Of the seven drugs, the solubility of fenofibrate and ticagrelor increased significantly in the mucus solution by 18-fold and 13-fold, respectively, as compared to the FaSSCoF. For hydrocortisone, prednisolone, and apixaban, solubility was 2 to3 times greater in the mucus solution. Current investigations are focusing on drug dissolution assessment within the artificial colonic mucus to complement the solubility measurements and increase our comprehension of the role of the mucus barrier as a dissolution compartment.

Acknowledgement:
The project is kindly supported by the VINNOVA funded competence center in drug delivery (The Swedish Drug Delivery Center).

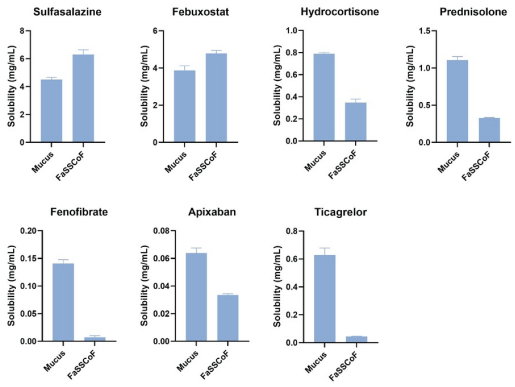

Figure 1. The solubility of sulfasalazine, febuxostat, hydrocortisone, prednisolone, fenofibrate, apixaban and ticagrelor in the artificial mucus solution and the FaSSCoF at 72 h (n = 3).

40 - Amorphous Powder for Systemic Nasal Delivery

Jonas Rudén[1] , *David Öhlund[1], Martin Jönsson[1], Erika Billinger[1], Robert Rönn[1], Jonas Sävmarker[1]*
[1] Orexo AB

Abstract text
INTRODUCTION

The nasal route of administration has several benefits for systemic drug delivery, including non-invasive dosing, rapid absorption and no first-pass metabolism. However, traditional liquid nasal sprays may have limitations, e.g: poor chemical stability in aqueous solutions, requires good solubility of the API, suboptimal and variable absorption due to swallowing.[1] We explored the possibility to develop a rapidly dissolving, spray-dried, nasal powder formulation to overcome limitations of liquid formulations while maintaining advantages of the nasal route of administration.

The particles are presented as an amorphous composite of the various ingredients providing for excellent chemical and physical stability, as well as rapid dissolution.

METHODS

A variety of different APIs ranging from small molecules up to attenuated virus have been successfully formulated into stable amorphous solid dispersions by spray drying. The powders were typically characterized by laser diffraction, XRPD, KF, HPLC (related substance, %RS) and the stability were followed up to 24 months at accelerated storage conditions (40°C/75%R.H.)[2]. Naloxone, nalmefene and epinephrine were all studied in separate cross-over, comparative, bioavailability studies in healthy volunteers (n=20 or 40). (Figure 1.)

RESULTS

The process yielded free flowing powders with a narrow particle size distribution, all between 10 – 80 µm ($D_{v,10}$ to $D_{v,90}$) and < 1% fines < 5 µm, i.e. particle size distribution suitable for effective nasal deposition[5]. The tested APIs were found chemically and physically stable at both long term and accelerated conditions (Figure 1.). In the comparative bioavailability studies, the powders were well tolerated and showed equivalent or superior onset time compared to the refences.

CONCLUSION

The combination of excellent stability with rapid dissolution in minimal amounts of liquid, seen for amorphOX®, are ideal properties for a nasally administered emergency medication.

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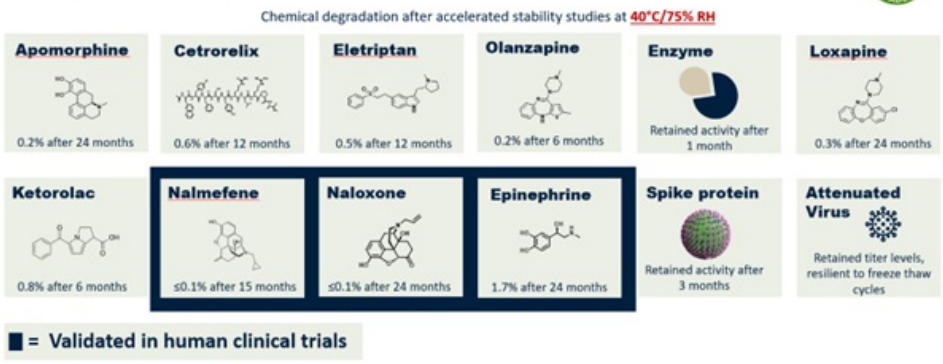
AmorphOX® – a versatile platform for intranasal drug delivery



41 - Amorphous sorafenib formulated using mesoporous magnesium carbonate exhibits 185% relative bioavailability in rodents

Jonas H Fagerberg[1] , *Khadijah Edueng*[1], *Tuulikki Lindmark*[1]
[1] Disruptive Pharma AB

Abstract text
The aim of this study was to compare the rodent bioavailability of a formulation based on sorafenib loaded onto mesoporous magnesium carbonate (MMC) with the bioavailability of Nexavar.

Sorafenib is a high dose, basic protein kinase inhibitor with poor aqueous solubility that may give rise to inter-patient variability with increased risk of adverse or lacking effects. MMC has an exceptionally high surface area and can be used to stabilize poorly soluble compounds in an amorphous state and thereby facilitate their absorption in the gastrointestinal tract.

Sorafenib was loaded onto MMC by dissolving the API in an organic solvent and mixing the solution with MMC before the solvent was evaporated. The product was characterized with regard to surface properties (nitrogen sorption), physical state (DSC), sorafenib contents (HPLC) prior to powder mixing. The MMC-sorafenib formulation and briefly milled comparator product was weighed in into gelatine capsules in doses equivalent to 0.8 mg/kg and 1.6 mg/kg respectively.

The capsules were given to the rats (n: 3 per group) and blood samples were withdrawn at defined timepoints up to 24 hours. The plasma samples were analyzed using HPLC-MS/MS after protein precipitation using acetonitrile with 1% formic acid.

The MMC-Sorafenib formulation gave rise to a C_{max} of 1100 ± 80 ng/mL and an AUC of 13000 ± 80 h*ng/ml, while the comparator gave rise to a C_{max} 1200 ± 500 ng/mL of and an AUC of 13800 ± 5400 h*ng/ml. The relative bioavailability of MMC-Sorafenib, calculated as the ratio of AUC divided by dose for the two formulations, was 185%

In conclusion the MMC-sorafenib formulation resulted in similar plasma concentration profiles as the comparator formulation despite administering only half dose. The increased absorption of amorphous sorafenib stabilized in MMC may be utilized to develop a potentially safer and more efficient drug product.

42 - An inverted cell culture model to study magnetic hyperthermia for the treatment of colorectal cancer

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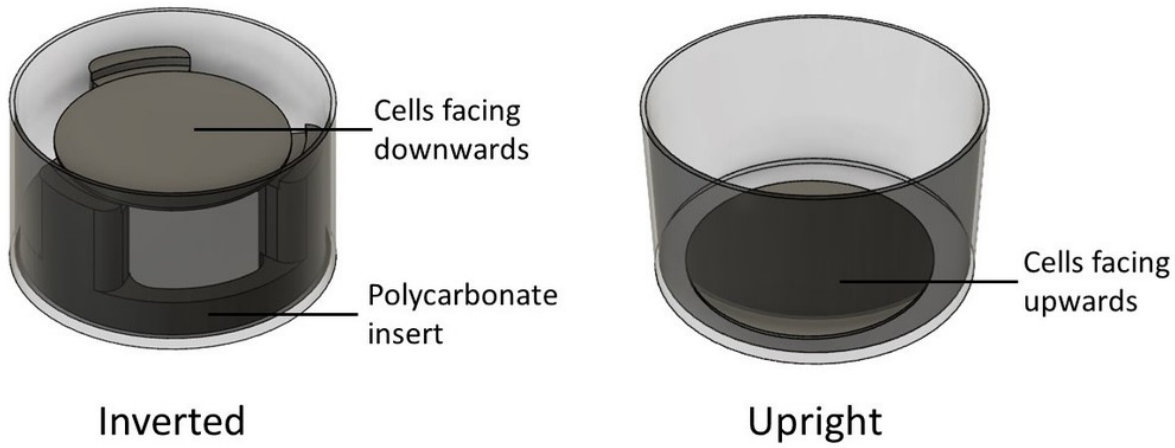
Abstract text
Colorectal cancer (CRC) is the third most common cancer worldwide. To enhance treatment outcomes, standard therapies like surgery, chemotherapy, and radiotherapy are often complemented with alternative approaches. Superparamagnetic iron oxide nanoparticles (SPIONs) induced magnetic hyperthermia therapy presents a promising alternative, offering advantages such as deep tumor penetration, local tumor heating, and targeted tumor destruction. This project focuses on developing SPIONs with high heating efficiency for oral delivery to colon cancer lesions. To assess the hyperthermia effects in a biosimilar environment, we introduced an inverted cell culture model. This novel approach overcomes the issue of excessive nanoparticle sedimentation observed in traditional upright cell configurations. Such sedimentation can significantly influence nanoparticle-cell interactions and magnetic hyperthermia performance, leading to localized overheating. In real colon tumor environments, nanoparticle sedimentation is unlikely to occur due to mucus barriers and bowel movements.

To address this, we developed colloidal stable PEGylated silica-coated SPIONs doped with zinc and manganese. The heating efficiency of these SPIONs was characterized in biosimilar colonic environments under an alternating magnetic field. Our findings revealed no significant differences in the heating efficiency of PEGylated particles across different colonic environments with varying viscosities. We then evaluated SPION uptake and hyperthermia outcomes using CRC cell lines Caco-2 and SW480 using the inverted and upright cell culture configurations (Figure 1). In the upright culture configuration, we observed substantial nanoparticle sedimentation and surface-bound SPIONs, while such phenomenon was not observed in the inverted culture configuration. These differences were quantitatively confirmed using Inductively Coupled Plasma Optical Emission Spectroscopy.

In summary, the inverted cell culture is a more realistic model for studying SPION-cell interactions when aiming at oral delivery of SPIONs to cancer sites, as it minimizes false positives arising from incomplete sedimentation removal.



43 - Click chemistry-based bioconjugation of iron oxide nanoparticles for diagnosis of inflammatory bowel disease

Shno Asad[1] , *Alexandra Teleki*[1]
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Abstract text
Introduction: Superparamagnetic iron oxide nanoparticles (SPION) are promising for targeted drug delivery and bioimaging. We have developed a single-step flame process for encapsulating SPION with a thin SiO_2 coating [1] that increases their biocompatibility and facilitates modification with e.g. antibodies by click chemistry. We hypothesize that by functionalizing SPIONs with ligands that target overexpressed biomarkers in the intestinal tissue, we can develop biosensors for monitoring inflammatory bowel disease (IBD) progression *in vivo* via magnetic resonance imaging (MRI).

Methods: SiO_2-coated SPIONs were modified by installing amine functional groups through silanization reaction using 3(aminopropyl)triethoxysilane and analyzed using Fourier Transform Infrared (FTIR) spectroscopy. Organic linkers with a terminal alkyne were either purchased or synthesized and installed onto the particle surface. ICAM-1 antibodies were modified with terminal azides using SiteClickTM Antibody Azido Modification Kit, followed by conjugation onto alkyne-modified SPIONs through click chemistry. Antibodies were tagged with fluorophores and visualized using fluorescence microscopy. Thermogravimetric analysis (TGA) was used to compare amount of organic material on SPION surface after each functionalization step. Targeting capability of the bioconjugated SPIONs are evaluated in vitro in an inflamed cell model and visualized with fluorescent microscopy and quantified using ICP-OES.

Results: The synthesized organic linker was obtained as a white solid (94 % yield), confirmed with nuclear magnetic resonance. Successful silanization and subsequent linker conjugation was confirmed with FTIR and TGA. Antibodies were successfully attached onto SPIONs via click chemistry and visualized with fluorescent microscopy. Quantification of iron content in inflammation induced Caco-2 cells showed significantly higher concentrations of ICAM1-conjugated particles compared to non-conjugated SPIONs.

Conclusions: Local diseases in the gastrointestinal tract (GIT), such as IBD, can be targeted using functionalized iron oxide nanoparticles. The magnetic properties of the particles make them useful for the development of an MRI-based diagnostic platform to detect and localize diseases in the GIT.

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44 - Engineering and in vivo validation of in situ forming hydrogels for the long-term treatment of glaucoma and choroidal neovascularization

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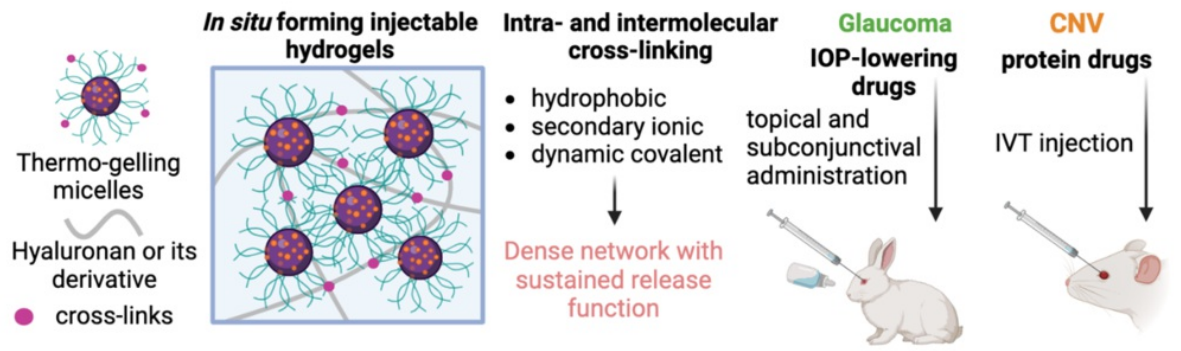
Abstract text

Novel approaches for the controlled release of therapeutics are needed to improve the clinical management of many ocular diseases. Currently, choroidal neovascularization is managed by monthly and bimonthly intravitreal injections of anti-VEGF antibodies, which constitutes a significant burden to patients and clinicians. Carbonic anhydrase inhibitors (CAIs) are used for the reduction of elevated intraocular pressure in managing glaucoma. Upon topical application, they have short elimination half-lives (a few hours) in the eye. The goal of this study is to design and evaluate new injectable hydrogels for sustained release of CAIs and anti-VEGF following topical, subconjunctival and intravitreal injections as shown in **Figure 1**. The hydrogels form *in situ* drug delivery depots that enable the long-acting topical application and intraocular injections with small needles. The density of the network is varied by utilizing the macromolecular interactions of different strengths, such as hydrophobic physical interactions, dynamic covalent bonds or secondary cross-linking. Thus, the hydrogel formulations with varying mechanical properties and sustained release function are evaluated in rabbit eyes for their safety and long-acting therapeutic effect after topical and subconjunctival injections.

More precisely, we developed a hydrogel based thermo-responsive copolymers and hyaluronan using a tandem dynamic and secondary cross-linking approaches to reach long-term drug release. Ionic strength of the formulation significantly changed mechanical properties and drug release of CAIs at large range of release rates. The release of antiVEGF proteins was sustained, the proteins remain their activity after 2 months of release *in vitro*. In preliminary animal studies, the material was injected to subconjunctival space in rabbits and hydrogel was formed in situ, constituting a delivery depot for controlled fluorescein release.



45 - Hyperthermia for local treatment of gynecological diseases using a microneedle-based drug delivery system

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Abstract text
Microneedles (MNs) address the limitations of conventional drug delivery systems by improving retention and targeting. The study aimed to develop dissolvable MNs with superparamagnetic iron oxide nanoparticles (SPIONs) to induce hyperthermia in gynecological tumors. SPIONs produce localized heat in an alternating magnetic field (AMF) through Néel or Brownian relaxation. This hyperthermia effect (at least 42°C) induces tumor-selective cytotoxicity, primarily affecting cancer cells (1). The combination of hyperthermia and chemotherapy enhances tumor sensitivity and treatment efficacy. Furthermore, MNs offer a precise, minimally invasive, and targeted strategy.

The fabrication of dissolvable MNs followed the procedures described (2). For the synthesis of SPIONs (manganese-doped) using flame spray pyrolysis, the technique outlined was employed (3). This study explored the effect of SPIONs on hyperthermia performance through dissolvable MN fabrication. MNs consist of polyvinyl alcohol to form water-soluble tips and a two-layered backing layer containing a polymethylmethacrylate (PMMA) layer with different SPION concentrations (3, 10, and 20 wt%) and a PMMA layer on top (Figure 1a,b). MNs without dissolvable tips but with 10 wt% SPION-loaded tips were also fabricated to compare. MagneTherm (Nanotherics Ltd.) was used to expose MNs to an AMF, capturing thermal images to monitor hyperthermia.

Both the soluble MNs (SPION content of 3, 10, and 20 wt%) and the MNs with insoluble SPION tips (10 wt%) showed hyperthermia effects. Specifically, the results indicated a temperature increase to 48°C after 3 minutes to a 14 mT AMF for the MNs with 3 wt% SPION content (Figure 1c). MNs with SPION-loaded tips showed higher heating than the MNs with soluble tips at a comparable SPION content due to the closer proximity of the SPIONs to the thermal camera.

Among the tested MNs, those with 3% SPION content and soluble tips reached a hyperthermia range of 48°C–52°C, close to the desired hyperthermia temperature.

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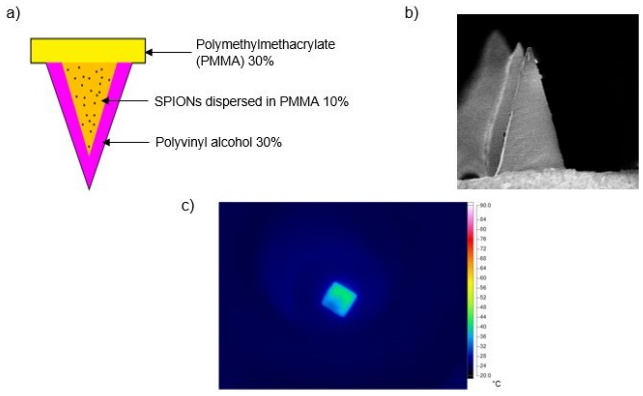

Figure 1. a) Schematic illustration of the fabrication process of dissolvable microneedles (MNs). b) Scanning Electron Microscopy (SEM) image of dissolvable MNs at 3% Superparamagnetic iron oxide nanoparticles (SPION) content. c) Hyperthermia effect in SPION-loaded MNs: Dissolvable MNs at 3% SPION content (maximum temperature reached: 48°C - 52°C).

46 - Impact of Simulated Intestinal Fluids on Dissolution, Solution Chemistry, and Membrane Transport of Amorphous Multidrug Formulations

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Abstract text

Understanding the drug behavior alone and in combination at the site of absorption is essential for efficient design of drug formulation. Gastrointestinal fluids contain several components which may have impact on solubility and transport of drugs across the intestinal membrane. In this study, amorphous multidrug formulations of two combinations: atazanavir and ritonavir (ATV-RTV) as well as felodipine and indapamide (FDN-IPM) were prepared together with polymer by solvent evaporation method using a rotary evaporator. The formulations were analyzed by Powder x-ray diffraction and differential scanning calorimetry. The dissolution (under non-sink conditions) and membrane transport in fasted state simulated intestinal fluid (FaSSIF) or buffer were investigated, and concentrations were determined by high performance liquid chromatography.

The formulations were confirmed to be amorphous by solid-state analysis. The amorphous solubility of the drugs was found to be enhanced to different extents by FaSSIF. In combination formulations, the maximum achievable concentration of drugs was reduced in FaSSIF (similar to buffer), but the extent of reduction was affected by the degree of FaSSIF solubilization. Dissolution studies of ATV and IPM revealed that the amorphous solubility of these two drugs was not affected by FaSSIF solubilization. In contrast, RTV was significantly affected by FaSSIF solubilization with a 30% reduction in the maximum achievable concentration upon combination with ATV, compared to 50% reduction in buffer. This positive deviation by FaSSIF solubilization was not reflected in the mass transport–time profiles. Interestingly, FDN concentrations remain constant until the amount of IPM added was over 1000 µg/mL. No decrease in the membrane transport of FDN was observed for a 1:1 molar ratio of FDN-IPM combination. This study demonstrates the importance of studying amorphous multidrug formulations under physiologically relevant conditions to obtain insights into the performance of these formulations after oral administration.

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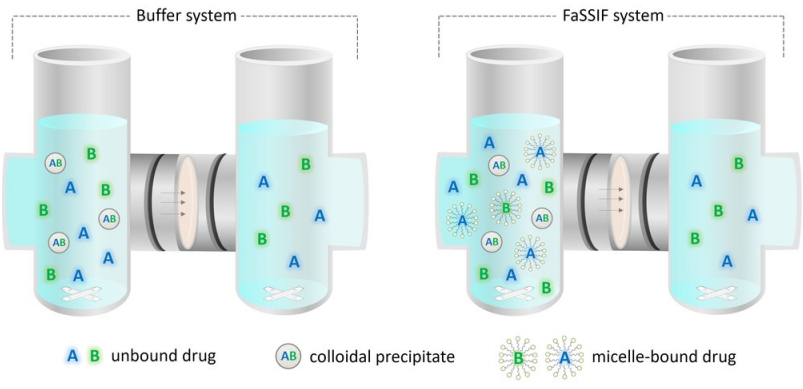

$$R_{(Solubility)} = \frac{Solubility_{(FaSSIF)}}{Solubility_{(Buffer)}} > 1 \qquad R_{(Flux)} = \frac{Flux_{(FaSSIF)}}{Flux_{(Buffer)}} \approx 1$$

47 - Impact on process parameters and solubility of main excipients on granule and tablet characteristics using a top sprayed fluid bed system

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[1] Department of Food Technology, Engineering and Nutrition, LTH, Lund, Sweden

Abstract text
Fluid bed granulation is a commonly used method in the pharmaceutical industry to increase compressibility and flowability of excipients and API´s. In the present research, we investigated the impact of process parameters and solubility of the main excipient on granule characteristics in a top sprayed fluid bed system. The results indicated that the size of the granules was more influenced by the spray rate compared to the flow of the fluidizing air. However, the solubility and characteristics of the filler used in the granule formulation had the biggest impact. Even when only a minor fraction of the filler was exchanged to a more soluble excipient, the resulting size of the granules was more different compared to increasing the spray rate. The granules where also compressed into tablets to determine compressibility and the effect of the granules on the characteristics of the resulting tablets. The result indicate that a higher spray rate gives a higher *in vivo* disintegration time, probably due to the larger granules. However, a more soluble filler increased the disintegration time.

48 - The Swedish Drug Delivery Center (SweDeliver)

Caroline Alvebratt[1] , *Alexandra Teleki*[1], *Christel Bergström*[1]
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Abstract text
SweDeliver is a world leading research and competence centre in Drug Delivery. Many novel molecules in the drug discovery and development pipeline are biological compounds such as proteins, peptides and RNA. These display problems of poor solubility, membrane permeability and low bioavailability. Thus, the Drug Delivery area is today facing tremendous challenges to develop new drug therapies.

SweDeliver was established in response to these challenges. Based on a multi-disciplinary collaboration between academia and industry with financial support from Vinnova (Sweden's Innovation Agency), the Faculty of Pharmacy at Uppsala University is the academic hub of the Centre with 17 industrial partners contributing expertise and research infrastructure as well as industrial perspectives and needs within the three main research areas of the Centre.

At the heart of SweDeliver, our PhD students and postdocs are working to solve scientific challenges defined in dialogue between the center's partners. With access to technology and guidance from prominent researchers in both academia and industry, they form an international constellation that conducts high-quality research, while laying the foundations for future leading positions in the Swedish life science sector.

SweDeliver has prioritized to focus on outstanding research challenges within

- Parenteral Drug Delivery
- Oral Drug Delivery
- Pulmonary Drug Delivery

Furthermore, SweDeliver specifically emphasizes training of its members in emerging techniques such as biological drugs and new modalities, computational modelling and simulation, and high-resolution analytics. These areas constitute strategic competences for the future drug delivery science.

The research will ultimately lead to development of new and improved drug therapies with respect to both efficacy and patient safety. The Centre will also further support the strong research environment and the drug delivery ecosystem in the Nordic region.

50 - NucleoDry – Research project for enhancing stability of gene therapies

Randi Nordström[1] , *Mehrnaz Shaali[1], Dileep Urimi[1], Agnes Zimmer[2], Klara Yngvesson[2], Pontus Blomberg[3], Jingyi Yan[4], Gustaf Ahlén[4], Matti Sällberg[4]*

[1] RISE Research Institutes of Sweden

[2] NorthX Biologics

[3] Vecura Karolinska university hospital

[4] Karolinska Institutet, Department of Laboratory Medicine

Abstract text

Vaccine formulations with demanding storage conditions has been a challenge for the world during the pandemic. Transportation and storage have required an advanced cold supply chain only available in the richest countries. Methods for producing vaccines with storage demands in fridge or higher temperatures would decrease the demands on transportation and storage and make the storage and distribution cheaper and more available world-wide. NucleoDry has the aim to develop production processes for manufacturing mRNA medicines that remain stable at 4 °C or higher, instead of the −80 °C that is most common today.

Lyophilization is a gentle technique for enhancing stability of biological modalities and enabling storage under milder conditions. There are challenges when drying lipid nanoparticles since water molecules is an integrated part of the particle structure. It is important to understand how removing water affects the nanostructure and also what the particle structure is after rehydration.

NucleoDry also has a goal to build up a new infrastructure for vaccine development to contribute to creating generic solutions for development of stable mRNA vaccines and pharmaceuticals. The project is being run by RISE together with Karolinska Institutet, the production unit Vecura at Karolinska University Hospital, and the Swedish innovation and manufacturing company NorthX Biologics. NucleoDry brings together expertise spanning the entire care chain from mRNA production via formulation development to scaling of manufacturing processes for large-scale pharmaceutical production.

51 - Effect of salts on the phase transitions in the sucrose-water system in relation to stabilization of biologics

Ekaterina Bogdanova[1, 2] , *Vitaly Kocherbitov*[1, 2], *Ben Humphreys*[3]

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[3] Institut Laue Langevin, Grenoble, France

Abstract text

The development of effective freeze-drying cycles for biologics requires an understanding of phase transitions in multicomponent non-equilibrium systems. The glass transition (Tg') of the amorphous part of the two-phase system and the onset of ice melting (Tm') are the key parameters for determination of the primary drying temperature[1] .

The purpose of this study is to investigate the effects of salts on the glass transition temperature and ice melting of frozen solutions of sucrose commonly used in freeze-drying of biologics. LiCl, NaCl, KCl, NaF, and NaI were chosen as one valence anion and cation salts from the Hofmeister series. Differential scanning calorimetry (DSC) was used to follow the phase behavior upon cooling and heating. The results show that the salts induce changes in the phase behavior of the sucrose-water system at sub-zero temperatures. A reduction of both Tg' and Tm' upon addition of salts is detected, which can be explained by the effects of salts on the positions of liquidus and Tg lines. LiCl is found to have the highest effect on the Tg'. The observed phenomenon is discussed in terms of thermodynamic (water activity), kinetic (water diffusion) and structural (size of the salt ions) perspectives.

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52 - Formulation factors affecting foam properties during vacuum foam-drying

Daniel Tristan Osanlóo[1, 2] , *Denny Mahlin*[1], *Simon Bjerregaard*[3], *Björn Bergenståhl*[2], *Anna Millqvist-Fureby*[1, 2]

[1] RISE

[2] Lunds Tekniska Högskola

[3] Ferring Pharmaceuticals A/S

Abstract text

Vacuum foam-drying is a drying technique which evaporates a solvent at reduced pressure and at ambient temperatures. By avoiding freezing (freeze-drying) and elevated temperatures (spray drying), vacuum foam-drying offers milder drying conditions which can benefit the stability of proteins (and other biologics). This work focuses on the principal influence vacuum foam-drying demonstrates on the formation of a solid foam and the surface formation.

A recombinant human bile salt stimulating lipase (lipase) was used as a model protein and formulated in a disaccharide matrix, with and without the presence of surface competitors. The lipase-formulations were dried by vacuum foam-drying which generated solid foams. The solid foams were analysed in terms of macrostructure and microstructure, by photographs and Scanning Electron Microscopy (SEM), respectively, where this presentation will highlight the morphological attributes. As surface exposures typically jeopardizes the functionality of active proteins, the chemical surface composition was analysed by X-ray Photon Spectroscopy (XPS) and related to the surface formation process.

Conducted trials showed intriguing differences in terms of how the excipients affected the formation of a solid foam. The macroscopic appearances were strongly affected by the choice of disaccharide, and to some extent by the choice of surfactant. The surface composition was related to disaccharide choice and the presence of a surfactant.

Vacuum foam-drying is a drying technique, which employed correctly introduces new possibilities for drying of proteins (and other biologics).

53 - Extracellular Vesicle-Inspired Asymmetric Vesicles for Nucleic Acid Delivery

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Abstract text

As natural carriers of biomolecules, extracellular vesicles (EVs) have great potential as drug delivery systems due to their biocompatibility, enhanced in vivo stability, weak immunogenicity, and ability to cross biological barriers. However, their heterogeneity and the challenges associated with their isolation, formulation, and scale-up pose limitations to their clinical translation. Hence, EV-inspired synthetic vesicles with asymmetric lipid distributions across the membrane bilayer were formulated in this study to mimic cell membranes and deliver nucleic acid for potential therapeutic applications. Asymmetric vesicles (aLUVs) composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) on the inner leaflet and 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) or brain sphingomyelin:cholesterol (SM:Chol) on the outer leaflet were successfully prepared using two methods – 1) methyl β-cyclodextrin-catalyzed lipid exchange and 2) enzymatic reaction with phospholipase D (PLD). Membrane asymmetry was then confirmed through zeta potential, NMR spectroscopy, and Amplex Red PLD assay, with 44.25-98% exchange efficiency. Afterwards, a splice-switching oligonucleotide (SSO) was encapsulated into the aLUVs and the loaded aLUVs were purified using size-exclusion chromatography. The lipid vesicles were found to be non-toxic to HeLa_Luc705 cells through Alamar Blue assay. Through fluorescence imaging, DOPC:SM:Chol aLUVs at 1:1 and 1:10 dilutions were successfully internalized by the cells and reached the nuclei after 4 hours, with increased uptake as compared to symmetric vesicles. These results were further supported by luciferase assay data, in which enzyme activity was observed in cells treated with 1:1 aLUVs after 4 and 24 hours, indicating the functional delivery of SSO cargo. Overall, EV-inspired aLUVs offer great potential as nanocarriers for nucleic acid delivery, leading towards diagnostic and therapeutic applications. Further studies involving more complex lipid compositions and protein receptors, other preparation methods, and a combination of quantitative techniques and high-resolution imaging to better understand cellular uptake and intracellular localization can also be explored.

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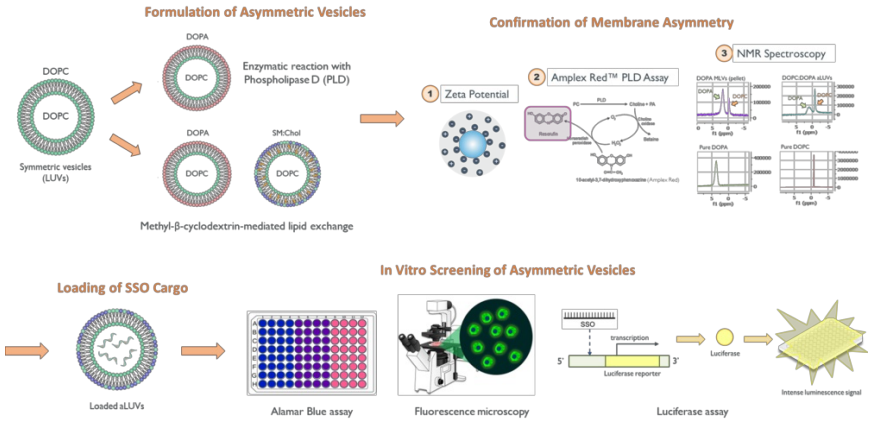

Figure 1. Schematic illustration of the research design.

54 - Versatile Gallium-based Nanoparticles as Antibacterial Agents

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[1] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet

Abstract text

Introduction

Antimicrobial resistance (AMR) has been identified as a global threat to public health by the WHO. It is estimated that, by 2050, AMR would lead to 10 million deaths annually[1]. However, the pace of developing new antibiotics lags significantly compared to the rate at which bacteria develop resistance. Therefore, it is valuable to develop novel antimicrobial agents that are independent from utilizing antibiotics.

Ferric ions (Fe^{3+}) are necessary for bacteria to maintain their metabolism and enzyme activity. Gallium ions (Ga^{3+}) share several similarities with Fe^{3+} in different manners, such as nuclear radius, coordination chemistry, and ionization potential, which makes them indistinguishable from Fe^{3+} by iron-binding proteins of bacteria[2]. Consequently, the introduction of exogenous Ga (III) can hinder bacterial metabolism and inhibit bacteria growth.

Method

Different sizes of gallium-based nanoparticles (GaNPs) were produced using flame spray pyrolysis (FSP)[3] and characterized by comprehensive solid-state characterization techniques to determine the physicochemical properties. These GaNPs were studied for their potential as antibacterial agents by analyzing the size-dependent effects on antibacterial efficacy. Furthermore, we loaded the antibacterial peptide, LL-37, onto the GaP NPs and assessed the loading efficiency through BCA assays.

Results and discussion

We produced GaNPs with 7 different sizes with FSP by controlling the flame conditions, which was validated by N_2 adsorption and TEM analysis. The specific surface area exhibited a correlation with the precursor concentration within the flame. In addition to their antimicrobial properties, GaNPs can act as drug carriers to deliver the antimicrobial peptide. While a synergistic effect may potentially enhance their bactericidal efficacy, further investigation is warranted.

Conclusion

In summary, GaNPs were synthesized using FSP. These NPs exhibit dual functionality, serving both as potent antibacterial agents and as versatile drug nanocarriers for delivering antibacterial peptides. Further experiments will provide the necessary framework for their clinical translation.

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55 - RealHOPE – Real World Handling of Protein Drugs: Exploration, Evaluation and Education

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Abstract text

Many new medicines are based on proteins, which have improved the lives of people with a range of diseases. The development, production, distribution, and handling of these drugs still present challenges that need to be addressed to generate further evidence on stability, and therefore safety and efficacy during handling in use.

The RealHOPE project focuses on creating an understanding of the real-life handling of protein drugs in hospital/community pharmacies, clinics, transportation and in the hands of patients. Our research covers in-use aspects of protein pharmaceuticals, investigating the critical steps where stability can be compromised. With the goal to support a safe and efficient use, we will develop tools for simulation of real-life events, mitigation of critical handling steps, and tailored education to ensure careful handling by healthcare and pharmacy staff as well as patients and caregivers.

The project will include measurements of real-life handling during transportation and by healthcare personnel and patients using smart tag technologies. The data will be used to develop new methods to simulate real-life events. The project also includes interviews with stakeholders to create a better picture on how protein drugs are handled today and how to educate stakeholders in the future.

RealHOPE is an IMI2-funded project that brings together 24 partners across EU, UK, Switzerland, and USA, representing major pharma companies, academia, hospital pharmacies and clinics, patient organisations as well as SMEs in logistics, analytical tools, and apps for education and disease monitoring. This presentation will introduce the project and the multidisciplinary approach to improve the use of protein drug products.

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www.realhope.se

56 - Intestinal organoid models for studying oral RNA therapeutics in Crohn's Disease

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Abstract text
Crohn’s disease (CD) is a chronic inflammatory disease which commonly affects the distal ileum and colon. CD is a non-communicable disease with an accelerating incidence globally (0.4–23 per 100,000 person/year)[1,2]. Despite advances in new immune modulators and biological treatment, up to 30% of patients become non-responders and suffer from disabling disease[1,3]. It is also recognized that the unmet clinical need associated with ileal CD is greater than with colonic CD, given the higher risk for delayed diagnosis, different pathophysiology and immune response, greater microbiota alterations, and higher rates of surgery[4]. Therefore, the need for novel, advanced therapies to suspend disease progression becomes more pressing than ever. GENEGUT, a European Research and Innovation Action, aims to transform the treatment of ileal CD by developing a first-in-class oral RNA-based therapy, tackling inflammation locally in the intestinal tissue, while avoiding systemic side effects. However, exploring these innovative therapeutic strategies to treat CD is largely hampered by a lack of models that reflect the complexity of the intestinal epithelium. Within this project, we aim to establish three-dimensional (3D) organoid models from human small intestinal tissue. Intestinal organoids partially recapitulate the identity of the original tissue *in vitro* and should therefore more accurately predict drug responses and serve as a platform for uptake studies. The polarity of the 3D intestinal organoids will be reversed, making them “apical-out” to facilitate accessing the apical surface of the epithelium, which normally interfaces with the external environment and uptakes drugs[5]. The intestinal organoids are profiled using compatible characterisation assays and functional studies are performed to evaluate the barrier properties. The established organoids will serve as preclinical tools and will be used to evaluate the gene modulation efficacy, demonstrate mechanism of cellular uptake as well as intracellular location of advanced oral RNA therapies developed within GENEGUT.

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57 - Particle Formation During Peristaltic Pumping of Therapeutic Proteins: Hofmeister Anion effects

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Abstract text

Pumping in the processing or administration of therapeutic proteins can involve several stress factors, like temperature changes, mechanical stress, and surface interactions, which can result in particle formation. In recent studies, protein adsorption and shedding of the formed protein film have been highlighted as the root cause of particle formation during pumping [1, 2]. Peristaltic pumping puts mechanical work on the tube to move the solution forward, allowing the protein to adsorb and release particles from the formed film continuously. We have studied the effects of different anions in the Hofmeister series on a monoclonal antibody during pumping to investigate particle formation mechanisms in relation to conformational stability and adsorption behavior. Anions affect solubility, stability, and interactions of proteins differently. In this study, SO_4^{2-}, Cl^-, and SCN^- are added in a concentration of 150 mM where the SCN^- has a salting in effect while SO_4^{2-} has a salting out effect and Cl^- is in the middle of the direct Hofmeister series. The results indicate that anions affect particle formation during pumping. The monomeric loss after pumping and thermal stability suggests that the protein stability follows in order of the direct Hofmeister series with the highest stability at SO_4^{2-} and lowest with SCN^-.

Unexpectedly, the presence of Cl^- led to significantly higher amounts of particles produced than SO_4^2 and SCN^-, possibly explained by a difference in adsorption behavior.

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58 - EXPERIENCES AND TRANSLATABILITY OF IN VITRO AND IN VIVO MODELS TO EVALUATE CAPRATE AS A PERMEATION ENHANCER

Prosper Emeh[1] , *Katarina Breitholtz*[2], *Staffan Berg*[3], *Charlotta Vedin*[2], *Constanze Hilgendorf*[2], *Nigel Davies*[3], *Christel Bergström*[1]

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Abstract text

Transient permeation enhancers (PEs) have been widely used to improve oral absorption of macromolecules. During pharmaceutical development, the correct selection of the macromolecule, PE and the combination needs to be made to maximize oral bioavailability and ensure successful clinical development. Various *in vitro* and *in vivo* methods have been investigated to optimize this selection. *In vitro* methods are generally preferred by the pharmaceutical industry to reduce the use of animals according to the “replacement, reduction and refinement” principle commonly termed “3Rs” and *in vitro* methods typically have a higher throughput. This poster compares two *in vitro* methods that are commonly used within the pharmaceutical industry, being Caco-2 cell monolayer and Ussing chamber to two *in vivo* models, being *in situ* intestinal instillation to rats and *in vivo* administration via an endoscope to pigs. All studies use solution formulation of sodium caprate which has been widely used as a PE and two macromolecules being FITC-dextran 4000 Da and MEDI7219, a GLP-1 receptor agonist peptide. The poster shares data using these models and highlights the challenges with the *in vitro* models in mimicking the processes occurring *in vivo*. The poster recommends a need to consider these differences when translating data generated using these *in vitro* models for evaluating macromolecules, PE and combinations thereof for enabling oral delivery.

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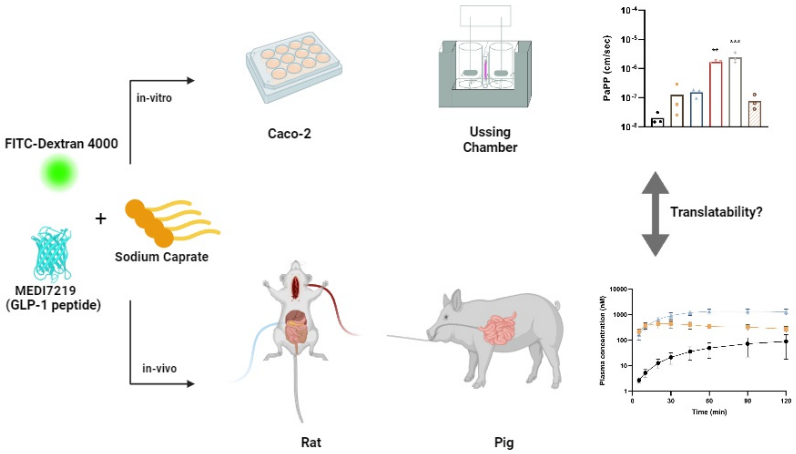

59 - Developing DNA-based hybrid carriers to control bacterial persistence and virulence in chronic skin wounds.

Sybil Obuobi[1] , *Alexandra Sousa*[1], *Natasa Skalko-Basnet*[1]

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Abstract text

DNA-based materials have enormous potential to advance the detection, prevention, and treatment of infectious diseases. However, their restricted biofilm penetration, poor affinity to bacteria and susceptibility to extracellular nucleases has stalled progress in their development. We capitalized on non-covalent interactions to develop lipid coated DNA nanocarriers and DNA micelles as hybrids, and studied their response against intracellular and/or biofilm infections. Lipid coating with a neutral lipid (i.e., soy phosphatidylcholine) enhanced loading efficiency of vancomycin, sustained drug release and improved killing of intracellular *S. aureus*[1]. By tuning the composition of the lipid corona, the hybrids exhibited pH-responsive properties that led to high biofilm accumulation and increased affinity to *S. aureus*[2]. The released DNA nanostructures (following rapid degradation of the hybrid by lipases) also disclosed potential anti-toxin activities. For hybrids with a lipid core (i.e., DNA micelles), incubation with *P. aeruginosa* biofilms revealed that the anionic surface did not restrict biofilm penetration[3]. For this system, co-assembly with polymyxin B led to the spontaneously formation of nanoparticles with significant structural stability and protection from dilution-induced disassembly. The observed safety, responsiveness and structural stability of the hybrids hint their possible applications in chronic wound management.

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60 - Diclofenac prodrugs nanoparticles: an alternative and efficient treatment for rheumatoid arthritis?

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Abstract text
We have synthesized lipidic prodrugs of diclofenac by grafting aliphatic chains (C10, C12, C16 and C18) to diclofenac through an ester bond. Their molecular formulas were confirmed through HR-MS and the formation of ester bond by FTIR. Nanoparticles of the different prodrugs were successfully formulated using emulsion evaporation method and DSPE-PEG2000 as the only excipient. All nanoparticles were spherical and had a size between 110 and 150 nm, PdI ≤ 0.2 and negative Zeta potential values from -30 to -50 mV. In addition, they were stable upon storage at 4°C up to 30-35 days. The encapsulation efficiency of the prodrug was above 90% independently of the aliphatic chain length grafted. Nanoparticles did not induce any toxicity on LPS-activated THP1 cells up to a concentration of 100 µg/mL (equivalent diclofenac) whereas diclofenac sodium salt IC50 was around 20 µg/mL. Following incubation of nanoparticles with LPS-activated THP1 cells, a dose dependent inhibition of TNF-α was observed comparable to standard diclofenac sodium. Based on in vitro studies representative nanoparticles “Prodrug 3 NPs” were selected for further in vitro and in vivo studies. Upon incubation in murine plasma, Prodrug 3 NPs underwent an enzymatic cleavage and almost 70 % of diclofenac was released from nanoparticles in 8 hours. In vivo studies on a collagen induced arthritis murine model showed contrasted results: on one hand Prodrug 3 NPs led to a significant decrease of arthritis score and of paw volume compared to PBS after the second injection, on the other hand the third injection induced an important hepatic toxicity with the death of half of the mice from the NP group. To promote the reduction of inflammation while avoiding hepatic toxicity using NPs would require to precisely study the No Observable Adverse Effect Level and the schedule of administration in the future.

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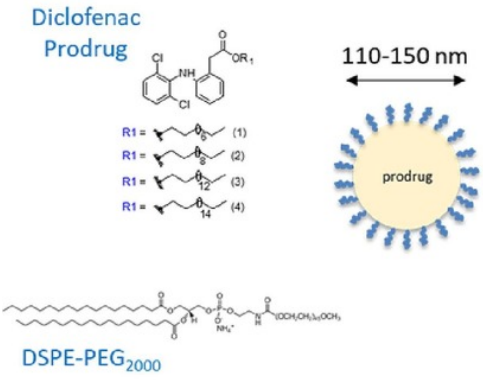
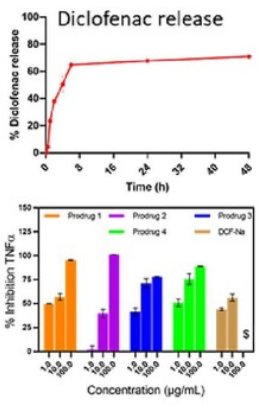
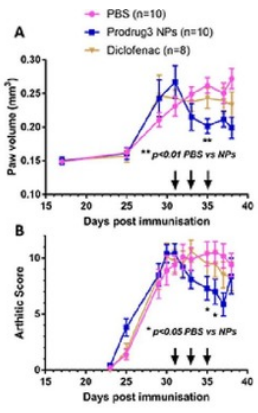




In vitro inhibition of TNFα



In vivo decrease of inflammation on CIA murine model

61 - Matrix-M™ a novel adjuvant for protein sub-unit vaccines

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Abstract text
The Matrix-M™ adjuvant technology has been developed by Novavax since late 1990. Novavax AB in Uppsala is responsible for research, development, and production of Matrix adjuvant for human and veterinary use. Matrix-M™ adjuvant is a self-assembled lipid nanoparticle system of plant derived saponins, phospholipids, and cholesterol and consists of different populations of physically stable nanoparticles mixed at a defined ratio. Figure 1 shows a model of the particle structure, revealing the unique spherical cage-like structure of Matrix-particles. The Matrix-M™ adjuvant is a key component of multiple novel vaccine products and candidates. It enhances and modulates the immunogenicity of antigens and enables the development of vaccines where traditional vaccines have been of limited efficacy or unsuccessful. Examples of vaccines with Matrix-M™ adjuvant are the NVX-CoV2373 Covid vaccine and the recent R21/ Matrix-M™ malaria vaccine authorized for use in Ghana, Nigeria, and Burkina Faso for children aged 5-36 months. Matrix-M™ does not require freezing temperatures which allows for safe distribution of vaccines also in developing countries. The poster will discuss the Matrix-M™ adjuvant components, formulation aspects and particle morphology, and biological function.

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Figure 1. Model of a Matrix particle with an indicative particle size of around 40-50 nm.

62 - The properties of gastric and intestinal mucus are affected differently when exposed to an oral permeation enhancer

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Abstract text

The oral permeation enhancer, sodium 8-[(2-hydroxybenzoyl)amino]octanoate (SNAC) increases the gastric absorption of the peptide semaglutide in the stomach (1). Yet in the small intestine, SNAC has been reported to increase viscosity of mucus when present (2), and hence may hamper intestinal absorption. Thus, the purpose of this study was to investigate in greater detail the effect of SNAC on gastric and intestinal mucus.

Effects of SNAC on the properties of *ex vivo* porcine intestinal mucus (PIM) and *ex vivo* gastric mucus (PGM) were evaluated by rheology, cryo-scanning electron microscopy (cryo-SEM), single particle tracking of fluorescent polystyrene nanoparticles (0.1-0.3 µm) and mucus permeability of the peptide cyclosporine A and the protein ovalbumin in the absence and presence of 150 mM SNAC.

The presence of SNAC significantly decreased permeation of cyclosporine A (~35 %) and ovalbumin (~50 %) through PIM, whereas the permeation through PGM was unaffected by the presence of SNAC. Interestingly, for PIM in presence of SNAC the viscosity increased 15-fold. Whereas the viscosity of PGM remained similar in the presence of SNAC. Cryo-SEM images of PIM in presence of SNAC revealed a tightened network within PIM, which correlated with 15-fold decrease in nanoparticle diffusion in PIM when exposed to SNAC (3).

The addition of the permeation enhancer SNAC increased the viscoelastic properties of PIM, which was found to be associated with decreased nanoparticle diffusion and a tighter network in PIM. In contrast, the viscoelastic properties of PGM were unaffected by the addition of SNAC, which suggests that SNAC-mucus interactions depend on the regional site in the gastrointestinal tract from which mucus was isolated and thus the type of mucin.

The Novo Nordisk Foundation (Grand Challenge Programme: NNF16OC0021948) is acknowledged for funding the work in the present study.

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